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The Approach to Antibodies After Heart Transplantation

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Abstract

a) Purpose of review—This review summarizes recent data about antibodies after cardiac transplantation; what testing modalities are available and how frequently to employ them; as well as when treatment is necessary.

b) Recent findings—Technologies available for antibody detection have progressed over the past couple decades. New and preformed antibodies are associated with worse outcomes in transplant recipients.

c) Summary—The frequency of screening for post-transplant antibodies and for antibody-mediated rejection (AMR) should be based on risk stratification. The presence of antibodies alone post-transplant does not constitute a diagnosis of AMR. Treatment of post-transplant antibodies and AMR should be made in conjunction with consideration of AMR grade and graft dysfunction. Future directions will involve improved detection methods and further understanding of non-HLA antibodies and de novo antibodies in the post-transplant population. Additionally, aggressive efforts are currently underway to provide more therapeutic options.

Keywords

cardiac transplantation; HLA antibodies; donor-specific antibodies (DSA); non-HLA antibodies; antibody-mediated rejection (AMR); antibody testing

Introduction

In the past couple decades, antibody-mediated rejection (AMR) has become recognized as the process that underlies hyperacute, early, and delayed rejection following cardiac allograft transplantation. It is associated with decreased survival and poor prognosis. However, management of antibodies after heart transplantation without clinical AMR is challenging and controversial. Specifically, there are prognostic differences whether the antibodies are de novo or persistent in a patient who was sensitized pre-transplant. In addition, technologies available for antibody detection have progressed over the past couple decades to provide

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Conflict of Interest

Olivia Gilbert and Patricia Chang declare no conflict of interest.

Compliance with Ethical Guidelines

Human and Animal Rights and Informed Consent

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better stratification for immunological risk. Whether this risk translates to poor outcomes such as AMR or biopsy-negative graft dysfunction is difficult to predict.

Accordingly, transplant physicians are faced with complex clinical scenarios with increasing frequency. Much of the objective evidence to guide management is limited to clinical data based on single-center experiences and consensus. This review summarizes recent data to address which antibodies are relevant to cardiac transplantation and AMR, what testing modalities are available, how frequently to employ them, and when treatment is necessary.

Antibodies Relevant to Cardiac Transplantation

Antibodies can be preformed in the “sensitized” recipient prior to cardiac transplantation or develop after transplant. These antibodies are commonly anti-human leukocyte antigen (HLA) antibodies, but they can also be non-HLA antibodies (1). At current time, HLA antibodies are relatively well-characterized and more easily detected than non-HLA antibodies (Table 1). This is reflective of the level of understanding of their respective antigens

Human leukocyte antigens derive from the major histocompatibility complex (MHC) located on Chromosome 6 and consist of self-recognizing cell surface molecules that allow the immune system to distinguish host from pathogen. The major classes of HLA relevant to solid organ transplant are I and II. Class I molecules include HLA-A, B, and C. Class II molecules include HLA DR, DP, and DQ. Antibodies that react to these antigens form antigen-antibody complexes along the allograft endothelial layer and cause activation of the complement cascade. This results in an inflammatory response characterized by increased vascular permeability, macrophage infiltration, microvascular thrombosis, and subsequent allograft dysfunction (2). While all nucleated cells express HLA Class I molecules, HLA Class II molecules are limited to antigen presenting cells such as B cells.

As part of the pre-transplant evaluation, potential transplant recipients are screened for anti-HLA antibodies to assess the patient’s immunologic risk for antibody-mediated rejection (AMR). Development of antibodies pre-transplant, termed allosensitization, results from antigenic exposure to foreign antigens. HLA antibodies often develop as a result of pregnancy, blood transfusion, or previous transplantation. Platelet transfusions have more of an antigenic impact than red blood cell transfusions, particularly with leukocyte-filtered products (3). Additionally relevant risk factors for allosensitization include specific HLA phenotypes (namely, HLA-DRB1*01 and HLA-DRB1*03), left ventricular assist device (LVAD) implantation, and viral infections, including hepatitis C and cytomegalovirus (CMV) (4, 5). The latter is particularly relevant for the development of the non-HLA antibodies, namely anti-endothelial cell antibodies, which have been shown to increase following CMV DNA detection (6). Regarding LVAD implantation, studies previously demonstrated that the HeartMate XVE device resulted in greater antibody production than the HeartMate II LVAD (7), although more recent reports suggest less difference by LVAD type (pulsatile versus continuous) with mixed results on clinical post-transplant outcomes (8–12).

When a transplant recipient has an antibody (old or new) against the donor's HLA antigen, this anti-HLA antibody is termed a donor specific antibody (DSA). Because DSA, particularly de novo antibodies, are associated with poor outcomes after heart transplant, it is important to understand differences in the development and detection of pre-existing versus de novo antibodies (13–16). There are two mechanisms that result in “new” expression of antibodies after transplant. The first involves cell memory in the pre-sensitized patient, whereby re-exposure to a previously recognized antigen results in a recall response with rapid antibody production early in the post-transplant period. HLA Class I antibodies are commonly the culprit DSA. The second is more delayed whereby the heart transplant recipient develops new DSA; these are frequently Class II antibodies. In order to screen for de novo antibodies, there is current recommendation for post-transplant testing for DSA within the first 90 days after transplantation (earlier and more frequently if the patient was sensitized), or when AMR is suspected (17). Of note, antibodies against HLA Class II antigens, particularly HLA-DQ antigens, are more likely to be persistent as well as to be associated with worse long-term outcomes, such as with cardiac allograft vasculopathy (CAV) and chronic rejection (14, 18–20).

Non-HLA antibodies should be considered as a cause of AMR if there is suspicion without evidence of HLA antibodies. The mechanism of non-HLA antibody-mediated cell destruction is different than HLA antibodies in that it is not complement-mediated (21). Major classes of non-HLA antibodies include the anti-cytoskeletal endothelial cell antibodies (e.g. vimentin, actin, cytokeratin, tubulin, and cytokeratin), major histocompatibility complex I chain-related antibodies A and B (MICA and MICB), angiotensin II-receptor 1 or angiotensin type 1 receptor (ATR-1 or AT₁R)-autoantibodies, endothelin receptor (ETR)-autoantibodies, and antibodies against glutathione S-transferase T1, cardiac myosin, and perlecan (17, 22–24) (Table 1). Even in the presence of HLA DSAs, concomitant non-HLA-specific antibodies can be prognostically deleterious (24). However, given their relative rare occurrence in AMR, non-HLA antibodies are not routinely assessed pre-transplant, nor post-transplant unless clinically indicated.

Antibody-mediated rejection (AMR)

In contrast to lymphocyte-mediated cellular rejection, AMR acts through antibodies to attack the donor graft. With improved antibody detection methods in immunopathology and immunohistochemistry, collaborative international efforts started with members of the International Society of Heart and Lung Transplantation (ISHLT) and the Banff Conferences on allograft pathology that resulted in standardization of its nomenclature and pathologic features in 2004 as well as further refinement of its immunopathologic diagnosis and clarification of varying clinical presentations and etiologies in 2010–2015 (25–30). Accordingly, the most current definition requires both histologic and immunopathologic evidence to support a diagnosis. In an effort to stratify the severity of disease with consideration of histologic and immunopathologic findings, the ISHLT consensus group defined four different categories of pathologic AMR (pAMR) in order to represent this spectrum (28). Thus, pAMR 0 suggests negativity (by both histopathology and immunopathology), pAMR 1 suggests suspicion (H+: histological findings present, immunopathologic findings negative; or I+: immunopathologic findings present, histological

findings negative), pAMR 2 suggests pathologic disease (both histological and immunopathologic findings present), and pAMR 3 suggests severe pathologic disease (immunopathology with severe histopathology).

Immunopathology is vital to the identification of AMR and requires special preparation of frozen biopsy samples for immunofluorescence. Immunopathologic requirements include evidence of immunoglobulin (IgG, IgM, or IgA) and complement deposition by immunofluorescence; staining of macrophages and/or complement by paraffin immunohistochemistry with immunoperoxidase staining; or appearance of fibrin in vessels (26). Histologic requirements for pAMR include evidence of capillary endothelial changes and macrophage infiltration. Severe histopathologic features include interstitial hemorrhage, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis and/or karyorrhexis, and marked edema. To diagnose pAMR, optimal staining by immunofluorescence should include both C3d and C4d, and immunoperoxidase staining of C4d is sufficient for immunohistochemistry.

While the presence of DSA is relevant for prognostication of outcomes as well as in supporting suspicion of AMR, it is not required for the diagnosis of AMR. In other words, the presence of DSA alone post-transplant does not constitute a diagnosis of pathologic or clinical AMR. Moreover, differentiating subclinical AMR (diagnosis by pathology only) from clinical AMR depends on the clinical scenario. The diagnosis and management of clinical AMR has been variable and recently summarized by a consensus conference in 2010 (27), a scientific statement from the American Heart Association in 2015 (17), and a consensus document for improving diagnostics for AMR from the XIIIth Banff Conference in 2015 (30). In addition to routine immunopathologic screening for AMR in the first year post-transplant, recommendations have been made to also screen for DSA.

Testing Modalities for Antibody Monitoring After Cardiac Transplantation

Screening for anti-HLA antibodies and post-transplant testing for DSAs include cell-based assays (complement-dependent cytotoxicity [CDC] and flow cytometry) and solid-phase immunoassays (enzyme-linked immunosorbent assays [ELISA] and polystyrene bead-based array assays). HLA antibody detection for pre-transplant screening was first managed with the cell-based CDC assays, which were relatively nonspecific and insensitive, followed by flow cytometry which was more sensitive. Subsequently, development of solid-phase immunoassays (SPI) resulted in even greater sensitivity in the detection of specific HLA antibodies, especially those that are donor-specific, for both pre- and post-transplant monitoring. Adjunctive techniques and assays should be considered to further characterize the potential clinical significance of detected antibodies.

Solid-phase immunoassays are the current technique for post-transplant antibody monitoring. Developed in the mid-1990s, this technique involves attachment of purified or recombinant HLA antigens to a carrier, such as an ELISA plate or plastic bead(s). A broad array of antigens can be attached to the carrier thereby enhancing the sensitivity of this assay. While ELISA provides a semiquantitative assessment of antibody binding, this technique is no longer used by HLA laboratories because of the superiority of bead-based

array assays for SPI, particularly those performed on the Luminex instrument (typically referred to as the Luminex assay) (31–33).

To briefly describe the Luminex bead-based assay, polystyrene beads are impregnated with different ratios of two fluorescent dyes (classifier signals) to yield up to 100 different bead populations. The antiglobulin reagent is labeled with a third fluorescent dye (the reporter signal) so that the fluorescence signature of each bead can be subsequently interrogated with a dual-laser instrument, to identify the bead population by one laser, and to simultaneously assess HLA-specific antibody binding via the reporter fluorescence. Antibody binding to the beads must be IgG antibodies since the fluorochrome-conjugated secondary (reporter) antibody to detect the binding is anti-human IgG and can be designed to have immunoglobulin-type or subclass specificity. Autoantibodies do not bind HLA coated beads in solid-phase assays, allowing the differentiation of HLA antibodies with clinical relevance. The three types of antigen panels used for bead-based assays include pooled antigen panels, phenotype panels, and single-antigen beads (SAB).

SAB arrays are the most sensitive and specific, whereby each bead population is coated with a molecule representing a single cloned allelic HLA class I or II antigen to determine the specific HLA antibodies in HLA antibody positive serum (34). Thus, SAB arrays provide the highest degree of HLA antibody resolution with a comprehensive array of common and many rare HLA alleles for all 11 HLA loci (HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPBA1, and DPB1).

HLA antibodies can be semi-quantified into broad categories (e.g., low or high) according to the mean fluorescence intensity (MFI) for the Luminex-based assays and the median channel of fluorescence (MCF) for flow cytometry crossmatch (FCXM). While antibody titer is the true quantitative measurement of antibody level, high titer and high MFI value are not synonymous. Most HLA labs use a MFI cutoff of between 1000 and 5000 for reporting as potentially clinically significant. But MFI level may not correlate with risk of AMR for a variety of reasons (35). These include interference from cryptic epitopes with HLA antibody as well as SAB inhibition from intrinsic and extrinsic factors (30).

To better understand the significance of detected HLA antibodies, modifications have been made to SPI in order to distinguish complementing-binding from non-complement binding antibodies, including the C4d, C3d, and C1q assays (36, 37). Among these, the C1q assay is considered the most clinically relevant because it is highly sensitive and also detects IgM complement-fixing antibodies. (38) Thus, it is prognostically important for identifying patients at high risk for poor outcomes and is available in many HLA laboratories (39). Nevertheless, the complement-binding assay in general has not yet been accepted as standard practice by all HLA laboratories because of its cost, and its dependency and correlation with antibody titer; thus, additional studies are required to establish its clinical role as a routine test (39).

Risk Stratification for Monitoring and Treatment

According to a 2013 Transplantation Society Consensus Guideline addressing antibody management after solid organ transplantation, frequency of monitoring should be based on risk stratification for developing AMR (34). Low-risk patients are those that are non-sensitized with a first transplant; intermediate-risk are those with prior HLA antibodies who are currently negative; high-risk are those with positive DSA but negative XM; and very high-risk are those who have been desensitized. Frequency of screening is fairly minimal in the low-risk patient (e.g., at least once between the 3rd and 12th month post-transplant, with changes in clinical status), and more frequently with increasing risk for AMR, as outlined in Figure 1.

In addition to those at intermediate risk for AMR with prior HLA antibodies, it has been suggested to also include patients treated with mechanical circulatory support such as LVADs, homografts for congenital heart defects, multiple transfusion, prior transplantation, and numerous prior childbirths in the intermediate category (12, 40, 41). These individuals should be monitored for DSA early post-transplant and, if present, frequently enough to determine persistence and stability with adjunctive testing (e.g., complement binding, antibody strength/titer, or Ig subclass) for further risk stratification if clinically indicated (13, 14, 19).

High-risk individuals should also include those who develop de novo DSA in addition to those who have positive DSA but negative XM. DSA should be screened relatively frequently with adjunctive testing as needed. Increased surveillance for rejection by biopsy and assessment of adequate immunosuppression should be considered, especially in the setting of isolated high risk DSA (e.g., high levels or titers, complement-binding, Class II antibodies, persistent antibodies). While very high-risk individuals should also undergo frequent monitoring, the difference in their management is a lower threshold for treatment (Figure 2).

Management of Antibodies

The management of antibodies before or after transplantation depends on the clinical scenario for which multiple agents have been used (Table 2). If the patient was sensitized pre-transplant, various immunomodulatory therapies are often considered pre-transplant and perioperatively (42). If the FCXM is positive, perioperative and early post-operative therapies are typically given (such as induction therapy and other adjunctive therapies). If antibodies persist post-transplant or develop de novo, therapy is tailored to the individual and clinical presentation.

Table 2 summarizes the various specific therapies that have been used to manage antibodies pre- or post-transplant and to treat AMR. Rituximab is a chimeric monoclonal antibody (mAB) against CD20 expressed on B cells that destroys cells via antibody-mediated cytotoxicity and complement fixation and activation. Basiliximab is a chimeric mAB against CD25 of the interleukin-2 (IL-2) receptor on T cells. By competing with IL-2 binding, T cell replication and activation of B cells is inhibited. Cyclophosphamide is an alkylating agent in

the nitrogen mustard family that interferes with DNA duplication and RNA transcription. Alemtuzumab is a humanized rabbit mAB targeted at CD52 Antigen (Ag), a human leukocyte differentiation Ag on the surface of most blood lymphocytes, macrophages, and monocytes. It kills cells antibody-mediated cytotoxicity and complement fixation and activation. Bortezomib is a selective 26S proteasome inhibitor, which results in apoptosis of plasma cells. Carfilzomib is a second-generation, irreversible 20S proteasome inhibitor that is an alternative to Bortezomib with an improved side-effect profile. Photophoresis involves treatment of a patient's leukocyte-rich plasma with a photosensitizing agent and ultraviolet A radiation prior to reinfusion. Affected leukocytes die over a 2-week period and cause a T-cell mediated suppressor response of other T cells (17, 43). Eculizumab is a mAB against IgG2/4, which targets and inhibits the complement cascade's terminal portion.

If DSAs are present in the setting of clinical AMR, treatment is indicated for AMR and the antibodies can be used as a marker for treatment response. Resolution of or decrease in DSA after treatment of AMR typically correlates with clinical improvement. Broad goals of treatment for AMR are discontinuation of cell injury and support for heart failure. Circulating antibodies can be removed with plasmapheresis. Residual levels can be suppressed with intravenous immunoglobulin (IVIG). Both B and T cells can be treated with corticosteroids; disease-modifying antirheumatic drugs (DMARD), such as alemtuzumab; or lymphoid irradiation (17). A more targeted T-cell therapy is photophoresis, and B-cell specific therapies include rituximab, cyclophosphamide, and splenectomy. Finally, modulation of complement activity can be achieved with eculizumab and IVIG. Additionally, individuals' maintenance immunosuppressive regimens can be adjusted such as transition of those on cyclosporine to tacrolimus as well as increased doses of mycophenolate mofetil and cyclosporine (44).

Thresholds of treatment for subclinical or pre-clinical AMR are more difficult to define, given that pathologic AMR can represent a spectrum (of sub-clinical, pre-clinical, acute clinical, and chronic disease process) as previously described, from pAMR 0 to pAMR 4 (45). Further organization of these groups by the presence or absence of graft dysfunction and DSA allow customization of treatment considerations (Figure 1) (17). Though there are no absolute recommendations regarding treatment, pAMR 3 is usually always treated as outcomes associated with it are otherwise grim, regardless of the presence of DSA or graft dysfunction.

Conclusions

New and preformed HLA DSAs are associated with worse outcomes in transplant recipients. In addition, non-HLA antibodies should be considered as a cause of AMR if there is suspicion without evidence of HLA antibodies. The frequency of screening for AMR should be based on risk stratification for development of AMR. Yet, the presence of antibodies alone post-transplant does not constitute a diagnosis of AMR, which is a histologic and immunopathologic diagnosis. Treatment of post-transplant antibodies should be made in conjunction with consideration of AMR grade and graft dysfunction.

Future directions will involve improved detection methods and further understanding of non-HLA antibodies and de novo antibodies in the post-transplant population. Additionally, aggressive efforts are currently underway to provide more therapeutic options, often modeled on treatments provided in abdominal transplant recipients, as we refine potential treatment algorithms. Potential new therapies for the cardiac transplant patient will likely include a C1 esterase inhibitor (C1-INH) and IdeS (a *Streptococcus pyogenes* proteolytic enzyme) that is currently being used in kidney transplant patients.

References

Papers of particular interest, published recently, have been highlighted as:

•Of importance

••Of major importance

1. Terasaki PI. Humoral theory of transplantation. *Am J Transplant*. 2003; 3(6):665–73. [PubMed: 12780557]
2. Brasile L, Zerbe T, Rabin B, Clarke J, Abrams A, Cerilli J. Identification of the antibody to vascular endothelial cells in patients undergoing cardiac transplantation. *Transplantation*. 1985; 40(6):672–5. [PubMed: 3907041]
3. Massad MG, Cook DJ, Schmitt SK, Smedira NG, McCarthy JF, Vargo RL, et al. Factors influencing HLA sensitization in implantable LVAD recipients. *Ann Thorac Surg*. 1997; 64(4):1120–5. [PubMed: 9354538]
4. Fuller TC, Fuller A. The humoral immune response against an HLA class I allodeterminant correlates with the HLA-DR phenotype of the responder. *Transplantation*. 1999; 68(2):173–82. [PubMed: 10440383]
5. Mehra MR, Uber PA, Uber WE, Scott RL, Park MH. Allosensitization in heart transplantation: implications and management strategies. *Curr Opin Cardiol*. 2003; 18(2):153–8. [PubMed: 12652223]
6. Möller E, Söderberg-Nauclér C, Sumitran-Karuppan S. Role of alloimmunity in clinical transplantation. *Rev Immunogenet*. 1999; 1(3):309–22. [PubMed: 11256422]
7. Arnaoutakis GJ, George TJ, Kilic A, Weiss ES, Russell SD, Conte JV, et al. Effect of sensitization in US heart transplant recipients bridged with a ventricular assist device: update in a modern cohort. *J Thorac Cardiovasc Surg*. 2011; 142(5):1236–45. 45.e1. [PubMed: 21839482]
8. Castleberry C, Zafar F, Thomas T, Khan MS, Bryant R, Chin C, et al. Allosensitization does not alter post-transplant outcomes in pediatric patients bridged to transplant with a ventricular assist device. *Pediatr Transplant*. 2016; 20(4):559–64. [PubMed: 27102953]
9. Alba AC, Tinckam K, Foroutan F, Nelson LM, Gustafsson F, Sander K, et al. Factors associated with anti-human leukocyte antigen antibodies in patients supported with continuous-flow devices and effect on probability of transplant and post-transplant outcomes. *J Heart Lung Transplant*. 2015; 34(5):685–92. [PubMed: 25638296]
10. Shankar N, Daly R, Geske J, Kushwaha SK, Timmons M, Joyce L, et al. LVAD implant as a bridge to heart transplantation is associated with allosensitization as measured by single antigen bead assay. *Transplantation*. 2013; 96(3):324–30. [PubMed: 23743727]
11. Kidambi S, Mohamedali B, Bhat G. Clinical outcomes in sensitized heart transplant patients bridged with ventricular assist devices. *Clin Transplant*. 2015; 29(6):499–505. [PubMed: 25773536]
12. Ko BS, Drakos S, Kfoury AG, Hurst D, Stoddard GJ, Willis CA, et al. Immunologic effects of continuous-flow left ventricular assist devices before and after heart transplant. *J Heart Lung Transplant*. 2016; 35(8):1024–30. [PubMed: 27316382]
13. Ho EK, Vlad G, Vasilescu ER, de la Torre L, Colovai AI, Burke E, et al. Pre- and posttransplantation allosensitization in heart allograft recipients: major impact of de novo

alloantibody production on allograft survival. *Hum Immunol.* 2011; 72(1):5–10. [PubMed: 20971146]

14. Smith JD, Banner NR, Hamour IM, Ozawa M, Goh A, Robinson D, et al. De novo donor HLA-specific antibodies after heart transplantation are an independent predictor of poor patient survival. *Am J Transplant.* 2011; 11(2):312–9. [PubMed: 21219570]
15. Tambur AR, Pamboukian SV, Costanzo MR, Herrera ND, Dunlap S, Montpetit M, et al. The presence of HLA-directed antibodies after heart transplantation is associated with poor allograft outcome. *Transplantation.* 2005; 80(8):1019–25. [PubMed: 16278580]
16. Kaczmarek I, Deutsch MA, Kauke T, Beiras-Fernandez A, Schmoeckel M, Vicol C, et al. Donor-specific HLA alloantibodies: long-term impact on cardiac allograft vasculopathy and mortality after heart transplant. *Exp Clin Transplant.* 2008; 6(3):229–35. [PubMed: 18954302]
- 17. Colvin MM, Cook JL, Chang P, Francis G, Hsu DT, Kiernan MS, 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(18):1608–39. This document encapsulates the history of our understanding of AMR and makes recommendations on diagnosis, surveillance, and management. [PubMed: 25838326]
18. Freitas MC, Rebellato LM, Ozawa M, Nguyen A, Sasaki N, Everly M, et al. The role of immunoglobulin-G subclasses and C1q in de novo HLA-DQ donor-specific antibody kidney transplantation outcomes. *Transplantation.* 2013; 95(9):1113–9. [PubMed: 23514959]
19. Irving CA, Carter V, Gennery AR, Parry G, Griselli M, Hasan A, et al. Effect of persistent versus transient donor-specific HLA antibodies on graft outcomes in pediatric cardiac transplantation. *J Heart Lung Transplant.* 2015; 34(10):1310–7. [PubMed: 26123951]
20. DeVos JM, Gaber AO, Knight RJ, Land GA, Suki WN, Gaber LW, et al. Donor-specific HLA-DQ antibodies may contribute to poor graft outcome after renal transplantation. *Kidney Int.* 2012; 82(5):598–604. [PubMed: 22622504]
- 21. Al-Mohaisen MA, Virani SA. Allosensitization in heart transplantation: an overview. *Can J Cardiol.* 2014; 30(2):161–72. This is a contemporary review of risk factors, testing, and management of allosensitization. [PubMed: 24373761]
22. Alvarez-Márquez A, Aguilera I, Blanco RM, Pascual D, Encarnación-Carrizosa M, Alvarez-López MR, et al. Positive association of anticytoskeletal endothelial cell antibodies and cardiac allograft rejection. *Hum Immunol.* 2008; 69(3):143–8. [PubMed: 18396205]
23. Barz D, Friedrich S, Schuller A, Rummler S. Antibodies against AT1-receptor in transplantation (diagnostics, treatment, clinical relevance). *Atheroscler Suppl.* 2015; 18:112–8. [PubMed: 25936314]
- 24. Reinsmoen NL, Lai CH, Mirocha J, Cao K, Ong G, Naim M, et al. Increased negative impact of donor HLA-specific together with non-HLA-specific antibodies on graft outcome. *Transplantation.* 2014; 97(5):595–601. This investigation demonstrates the negative impact of both HLA and non-HLA de novo antibodies on cardiac graft outcomes. [PubMed: 24162250]
25. Stewart S, Winters GL, Fishbein MC, Tazelaar HD, Kobashigawa J, Abrams J, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant.* 2005; 24(11):1710–20. [PubMed: 16297770]
26. Reed EF, Demetris AJ, Hammond E, Itescu S, Kobashigawa JA, Reinsmoen NL, et al. Acute antibody-mediated rejection of cardiac transplants. *J Heart Lung Transplant.* 2006; 25(2):153–9. [PubMed: 16446213]
27. Kobashigawa J, Crespo-Leiro MG, Ensminger SM, Reichenspurner H, Angelini A, Berry G, et al. Report from a consensus conference on antibody-mediated rejection in heart transplantation. *J Heart Lung Transplant.* 2011; 30(3):252–69. [PubMed: 21300295]
- 28. Berry GJ, Burke MM, Andersen C, Bruneval P, Fedrigo M, Fishbein MC, et al. The 2013 International Society for Heart and Lung Transplantation Working Formulation for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation. *J Heart Lung Transplant.* 2013; 32(12):1147–62. This document provided updates to the 2010 ISHLT consensus document with the reducing variation in the diagnosis of AMR in order to improve algorithms for therapy. [PubMed: 24263017]

29. Berry GJ, Angelini A, Burke MM, Bruneval P, Fishbein MC, Hammond E, et al. The ISHLT working formulation for pathologic diagnosis of antibody-mediated rejection in heart transplantation: evolution and current status (2005–2011). *J Heart Lung Transplant*. 2011; 30(6): 601–11. [PubMed: 21555100]
- 30. Bruneval P, Angelini A, Miller D, Potena L, Loupy A, Zeevi A, et al. The XIIIth Banff Conference on Allograft Pathology: The Banff 2015 Heart Meeting Report: Improving Antibody-Mediated Rejection Diagnostics: Strengths, Unmet Needs, and Future Directions. *Am J Transplant*. 2017; 17(1):42–53. In addition to analyzing the strengths and weakness of the current rejection grading system, detection methods, and management, this documenton suggests standardization of our approach to CAV. [PubMed: 27862968]
31. Zachary AA, Ratner LE, Graziani JA, Lucas DP, Delaney NL, Leffell MS. Characterization of HLA class I specific antibodies by ELISA using solubilized antigen targets: II. Clinical relevance. *Hum Immunol*. 2001; 62(3):236–46. [PubMed: 11250041]
32. Pei R, Lee J, Chen T, Rojo S, Terasaki PI. Flow cytometric detection of HLA antibodies using a spectrum of microbeads. *Hum Immunol*. 1999; 60(12):1293–302. [PubMed: 10626745]
33. Pei R, Lee JH, Shih NJ, Chen M, Terasaki PI. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. *Transplantation*. 2003; 75(1):43–9. [PubMed: 12544869]
- 34. Tait BD, Süsal C, Gebel HM, Nickerson PW, Zachary AA, Claas FH, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation*. 2013; 95(1):19–47. In the face of increased sensitivity and specificity available with SPIs, this document defined low-, intermediate-, and high-risk patients based on allosensitization risk to warrant frequency of antibody testing. [PubMed: 23238534]
- 35. Ellis TM. Interpretation of HLA single antigen bead assays. *Transplant Rev (Orlando)*. 2013; 27(4):108–11. This is a review of our current understanding the benefits and limitations intrinsic to SAB assays for the detection and quantitation of HLA antibodies. [PubMed: 23958238]
36. Smith JD, Hamour IM, Banner NR, Rose ML. C4d fixing, luminex binding antibodies - a new tool for prediction of graft failure after heart transplantation. *Am J Transplant*. 2007; 7(12):2809–15. [PubMed: 17908268]
37. Chin C, Chen G, Sequeria F, Berry G, Siehr S, Bernstein D, et al. Clinical usefulness of a novel C1q assay to detect immunoglobulin G antibodies capable of fixing complement in sensitized pediatric heart transplant patients. *J Heart Lung Transplant*. 2011; 30(2):158–63. [PubMed: 20951058]
38. Llorente S, Boix F, Eguia J, López M, Bosch A, Martinez H, et al. C1q-fixing human leukocyte antigen assay in immunized renal patients: correlation between Luminex SAB-C1q and SAB-IgG. *Transplant Proc*. 2012; 44(9):2535–7. [PubMed: 23146446]
39. Zeevi A, Lunz J, Feingold B, Shullo M, Bermudez C, Teuteberg J, et al. Persistent strong anti-HLA antibody at high titer is complement binding and associated with increased risk of antibody-mediated rejection in heart transplant recipients. *J Heart Lung Transplant*. 2013; 32(1):98–105. [PubMed: 23142561]
40. Kaufman BD, Shaddy RE. Immunologic considerations in heart.
- 41. Guichard-Romero A, Marino-Vazquez LA, Castelán N, López M, González-Tableros N, Arvizu A, et al. Impact of pretransplant exposure to allosensitization factors generating HLA antibodies in the Luminex era. *Transpl Immunol*. 2016; 38:33–9. This study used Luminex measurement of PRA to assess attributable risk of sensitizing factors. [PubMed: 27553730]
- 42. Chih S, Patel J. Desensitization strategies in adult heart transplantation-Will persistence pay off? *J Heart Lung Transplant*. 2016; 35(8):962–72. This review provides an overview of current desensitization strategies, limitations, and future directions. [PubMed: 27216504]
43. Marques MB, Schwartz J. Update on extracorporeal photopheresis in heart and lung transplantation. *J Clin Apher*. 2011; 26(3):146–51. [PubMed: 21647952]
44. Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S, et al. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant*. 2010; 29(8):914–56. [PubMed: 20643330]

45. Chih S, Tinckam KJ, Ross HJ. A survey of current practice for antibody-mediated rejection in heart transplantation. *Am J Transplant*. 2013; 13(4):1069–74. [PubMed: 23414257]
46. Leech SH, Lopez-Cepero M, LeFor WM, DiChiara L, Weston M, Furukawa S, et al. Management of the sensitized cardiac recipient: the use of plasmapheresis and intravenous immunoglobulin. *Clin Transplant*. 2006; 20(4):476–84. [PubMed: 16842525]
47. Kobashigawa JA, Patel JK, Kittleson MM, Kawano MA, Kiyosaki KK, Davis SN, et al. The long-term outcome of treated sensitized patients who undergo heart transplantation. *Clin Transplant*. 2011; 25(1):E61–7. [PubMed: 20973825]
48. Patel J, Everly M, Chang D, Kittleson M, Reed E, Kobashigawa J. Reduction of alloantibodies via proteasome inhibition in cardiac transplantation. *J Heart Lung Transplant*. 2011; 30(12):1320–6. [PubMed: 21968130]
49. Bu in D, Gustafsson R, Ekmehag B, Kornhall B, Algotsson L, Lund U, et al. Desensitization and heart transplantation of a patient with high levels of donor-reactive anti-human leukocyte antigen antibodies. *Transplantation*. 2010; 90(11):1220–5. [PubMed: 20885338]
50. Daly KP, Chandler SF, Almond CS, Singh TP, Mah H, Milford E, et al. Antibody depletion for the treatment of crossmatch-positive pediatric heart transplant recipients. *Pediatr Transplant*. 2013; 17(7):661–9. [PubMed: 23919762]
51. Jackups R, Canter C, Sweet SC, Mohanakumar T, Morris GP. Measurement of donor-specific HLA antibodies following plasma exchange therapy predicts clinical outcome in pediatric heart and lung transplant recipients with antibody-mediated rejection. *J Clin Apher*. 2013; 28(4):301–8. [PubMed: 23426730]
52. Larson DF, Elkund DK, Arabia F, Copeland JG. Plasmapheresis during cardiopulmonary bypass: a proposed treatment for presensitized cardiac transplantation patients. *J Extra Corpor Technol*. 1999; 31(4):177–83. [PubMed: 10915474]
53. Lick SD, Beckles DL, Piovesana G, Vaidya S, Indrikovs A, Barbagelata NA, et al. Transplantation of high panel-reactive antibody left ventricular assist device patients without crossmatch using on-bypass pheresis and alemtuzumab. *Ann Thorac Surg*. 2011; 92(4):1428–34. [PubMed: 21855854]
54. Holt DB, Lublin DM, Phelan DL, Boslaugh SE, Gandhi SK, Huddleston CB, et al. Mortality and morbidity in pre-sensitized pediatric heart transplant recipients with a positive donor crossmatch utilizing peri-operative plasmapheresis and cytolytic therapy. *J Heart Lung Transplant*. 2007; 26(9):876–82. [PubMed: 17845925]
55. Pollock-BarZiv SM, den Hollander N, Ngan BY, Kantor P, McCrindle B, West LJ, et al. Pediatric heart transplantation in human leukocyte antigen sensitized patients: evolving management and assessment of intermediate-term outcomes in a high-risk population. *Circulation*. 2007; 116(11 Suppl):II172–8. [PubMed: 17846300]
56. Schumacher KR, Ramon DS, Kamoun M, Caruthers R, Gajarski RJ. HLA desensitization in pediatric heart transplant candidates: efficacy of rituximab and IVIg. *J Heart Lung Transplant*. 2012; 31(9):1041–2. [PubMed: 22748817]
57. John R, Lietz K, Burke E, Ankersmit J, Mancini D, Suciu-Foca N, et al. Intravenous immunoglobulin reduces anti-HLA alloreactivity and shortens waiting time to cardiac transplantation in highly sensitized left ventricular assist device recipients. *Circulation*. 1999; 100(19 Suppl):II229–35. [PubMed: 10567309]
58. Pisani BA, Mullen GM, Malinowska K, Lawless CE, Mendez J, Silver MA, et al. Plasmapheresis with intravenous immunoglobulin G is effective in patients with elevated panel reactive antibody prior to cardiac transplantation. *J Heart Lung Transplant*. 1999; 18(7):701–6. [PubMed: 10452347]
59. Richmond ME, Hsu DT, Mosca RS, Chen J, Quaegebeur JM, Addonizio LJ, et al. Outcomes in pediatric cardiac transplantation with a positive HLA cross-match. *Pediatr Transplant*. 2012; 16(1):29–35. [PubMed: 22017728]
60. Weeks P, Sieg A, Ling M, Bai Y, Nathan S, Rajapreyar I. Carfilzomib-Rituximab Desensitization in Sensitized Heart Transplant Candidates: A Single Center Experience. *The Journal of Heart and Lung Transplantation*. 2016
61. Bonaros N, Dunkler D, Kocher A, Imhof M, Grimm M, Zuckermann A, et al. Ten-year follow-up of a prospective, randomized trial of BT563/bb10 versus anti-thymocyte globulin as induction

- therapy after heart transplantation. *J Heart Lung Transplant*. 2006; 25(9):1154–63. [PubMed: 16962480]
62. Carrier M, Leblanc MH, Perrault LP, White M, Doyle D, Beaudoin D, et al. Basiliximab and rabbit anti-thymocyte globulin for prophylaxis of acute rejection after heart transplantation: a non-inferiority trial. *J Heart Lung Transplant*. 2007; 26(3):258–63. [PubMed: 17346628]
 63. De Santo LS, Della Corte A, Romano G, Amarelli C, Onorati F, Torella M, et al. Midterm results of a prospective randomized comparison of two different rabbit-antithymocyte globulin induction therapies after heart transplantation. *Transplant Proc*. 2004; 36(3):631–7. [PubMed: 15110616]
 64. Emin A, Rogers CA, Thekkudan J, Bonser RS, Banner NR, Steering Group UKCTA. Antithymocyte globulin induction therapy for adult heart transplantation: a UK national study. *J Heart Lung Transplant*. 2011; 30(7):770–7. [PubMed: 21444210]
 65. Faggian G, Forni A, Milano AD, Chiominto B, Walpoth BH, Scarabelli T, et al. Antithymocyte globulin induction therapy in heart transplantation: prospective randomized study of high vs standard dosage. *Transplant Proc*. 2010; 42(9):3679–87. [PubMed: 21094838]
 66. Koch A, Daniel V, Dengler TJ, Schnabel PA, Hagl S, Sack FU. Effectivity of a T-cell-adapted induction therapy with anti-thymocyte globulin (Sangstat). *J Heart Lung Transplant*. 2005; 24(6):708–13. [PubMed: 15949731]
 67. Krogsgaard K, Boesgaard S, Aldershvile J, Arendrup H, Mortensen SA, Petterson G. Cytomegalovirus infection rate among heart transplant patients in relation to the potency of antithymocyte immunoglobulin induction therapy. Copenhagen Heart Transplant Group. *Transplant Proc*. 1994; 26(3):1718. [PubMed: 8030099]
 68. Mattei MF, Redonnet M, Gandjbakhch I, Bandini AM, Billes A, Epailly E, et al. Lower risk of infectious deaths in cardiac transplant patients receiving basiliximab versus anti-thymocyte globulin as induction therapy. *J Heart Lung Transplant*. 2007; 26(7):693–9. [PubMed: 17613399]
 69. Ladowski JS, Dillon T, Schatzlein MH, Peterson AC, Deschner WP, Beatty L, et al. Prophylaxis of heart transplant rejection with either antithymocyte globulin-, Minnesota antilymphocyte globulin-, or an OKT3-based protocol. *J Cardiovasc Surg (Torino)*. 1993; 34(2):135–40.
 70. Yamani MH, Taylor DO, Czerr J, Haire C, Kring R, Zhou L, et al. Thymoglobulin induction and steroid avoidance in cardiac transplantation: results of a prospective, randomized, controlled study. *Clin Transplant*. 2008; 22(1):76–81. [PubMed: 18251036]
 71. Zhang R, Haverich A, Strüder M, Simon A, Bara C. Delayed onset of cardiac allograft vasculopathy by induction therapy using anti-thymocyte globulin. *J Heart Lung Transplant*. 2008; 27(6):603–9. [PubMed: 18503958]
 72. Beniaminovitz A, Itescu S, Lietz K, Donovan M, Burke EM, Groff BD, et al. Prevention of rejection in cardiac transplantation by blockade of the interleukin-2 receptor with a monoclonal antibody. *N Engl J Med*. 2000; 342(9):613–9. [PubMed: 10699160]
 73. Delgado DH, Miriuka SG, Cusimano RJ, Feindel C, Rao V, Ross HJ. Use of basiliximab and cyclosporine in heart transplant patients with pre-operative renal dysfunction. *J Heart Lung Transplant*. 2005; 24(2):166–9. [PubMed: 15701432]
 74. Rosenberg PB, Vriesendorp AE, Drazner MH, Dries DL, Kaiser PA, Hynan LS, et al. Induction therapy with basiliximab allows delayed initiation of cyclosporine and preserves renal function after cardiac transplantation. *J Heart Lung Transplant*. 2005; 24(9):1327–31. [PubMed: 16143252]
 75. Segovia J, Rodríguez-Lambert JL, Crespo-Leiro MG, Almenar L, Roig E, Gómez-Sánchez MA, et al. A randomized multicenter comparison of basiliximab and muromonab (OKT3) in heart transplantation: SIMCOR study. *Transplantation*. 2006; 81(11):1542–8. [PubMed: 16770243]
 76. Itescu S, Burke E, Lietz K, John R, Mancini D, Michler R, et al. Intravenous pulse administration of cyclophosphamide is an effective and safe treatment for sensitized cardiac allograft recipients. *Circulation*. 2002; 105(10):1214–9. [PubMed: 11889016]
 77. Lick SD, Vaidya S, Kollar AC, Boor PJ, Vertrees RA. Peri-operative alemtuzumab (Campath-1H) and plasmapheresis for high-PRA positive lymphocyte crossmatch heart transplant: a strategy to shorten left ventricular assist device support. *J Heart Lung Transplant*. 2008; 27(9):1036–9. [PubMed: 18765198]

78. Patel L, Dilibero D, Kittleson M, Sana S, Liou F, Chang D, et al. Terminal Complement Inhibition for Highly Sensitized Patients Undergoing Heart Transplant - Doable? : The Journal of Heart and Lung Transplant. 2015

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Figure 1.
Stratification for DSA screening based on AMR risk. Adapted from Tait *et al* (34).

Figure 2.

Treatment options for pAMR based on the presence of donor specific antibody (DSA) and dysfunctional graft (DG). Green indicates that no treatment should be considered, yellow indicates that treatment could be considered based on clinical circumstances, and red indicates that treatment should be done regardless of clinical status. Adapted from Colvin *et al* (17).

Abbreviations: AMR=antibody-mediated rejection, DG=dysfunction graft, DSA=donor-specific antibody, pAMR=ISHLT pathologic grade of AMR, XM=crossmatch

Table 1

Types of Post-Transplant Antibodies.

POST-TRANSPLANT ANTIBODIES Preformed or De novo		
HLA <i>Complement dependent</i>		Non-HLA <i>Complement independent</i>
DSA	Non-DSA	
	Exposure-Related	1 Anti-cytoskeletal antibodies (Vimentin, Actin, Cytokeratin, Tubulin, Cytokeratin) 2 Major Histocompatibility complex I chain-related antibodies A and B (MICA and MICB) 3 Angiotensin II-receptor 1 or angiotensin type 1 receptor-autoantibodies (ATR-1, AT ₁ R) 4 Endothelin receptor-autoantibodies (ETR) 5 Miscellaneous antibodies against glutathione S-transferase T1, cardiac myosin, and perlecan.
	1 Pregnancy	
	2 Blood transfusion	
	3 Previous transplant	
	Miscellaneous	
	1 HLA Phenotype	
	2 LVAD	
	3 Viral infections (HCV, CMV)	
	4 Ethnicity	

Abbreviations: DSA=donor-specific antibody, HLA=human leukocyte antigen, LVAD=Left ventricular assist device.

Table 2

Antibody management

Therapy	Pre-Transplant (Desensitization)	Intra-Operative (Desensitization)	Post-Operative (Desensitization or XM-Positive)	AMR(17)
Corticosteroids		+ (<i>per institutional protocol</i>)	+ (<i>per institutional protocol</i>)	+
Plasmapheresis (or Immunoabsorption)	+ (46–48)	+ (49–54)	+ (50, 51, 54, 55)	+
IVIG	+ (46, 47, 56, 57)		+ (47–50, 58, 59)	+
Rituximab	+ (47, 56, 60)		+ (55, 59)	+
Rabbit anti-thymocyte globulin			+ (<i>induction</i>) (61–71)	+
Basiliximab			+ (<i>induction</i>) (62, 68, 72–75)	
Cyclophosphamide	+ (76)		+ (54)	+
Alemtuzumab			+ (53, 77)	+
Bortezomib	+ (48)			+
Carfilzomib	+ (60)			
Photopheresis			+ (54)	+
Eculizumab		+ (78)	+ (78)	+