Challenges with sensitized recipients in pediatric heart transplantation

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The sensitization of patients to human leukocyte antigens prior to heart transplantation is increasingly being recognized as an important challenge both before and after the transplant, and the effects of sensitization on clinical outcomes are just beginning to be understood. Many patients are listed with the requirement of a negative prospective or virtual crossmatch prior to accepting a donor organ. This strategy has been associated with both longer waitlist times and higher waitlist mortality. An alternative approach is to transplant across a potentially positive crossmatch while utilizing strategies to decrease the significance of the human leukocyte antigen antibodies. This review will examine the challenges and the impact of sensitization on pediatric patients prior to and following heart transplantation.

KEYWORDS: Sensitization; Crossmatch; Heart; Transplant; Pediatrics; Panel Reactive Antibodies; Human Leukocyte Antigen (HLA).

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INTRODUCTION

The production of antibodies to human leukocyte antigen (HLA) prior to transplantation is increasingly being recognized as an important contributor to clinical outcomes in all solid organ transplants. The role of the anti-HLA antibodies was first recognized in kidney transplantation over 40 years ago, and knowledge of their significance spread to the transplantation of other solid organs in subsequent years (1). This review will primarily focus on the issues associated with sensitization in pediatric heart transplantation, with some references to the other solid organ transplants. Although a detailed description of the development of these antibodies and the methods used for screening is beyond the scope of this chapter, a framework will be provided on which the rest of the discussion will occur. For readers interested in further details on this topic, there are some excellent recently published reviews (2,3).

ANTI-HLA ANTIBODIES

Anti-HLA antibodies are antibodies directed against antigens on Class I and Class II major histocompatibility complexes. Class I molecules are found on all nucleated cells in the body, and Class II expression is observed predominantly on antigen-presenting cells and activated endothelial cells (3-5). Anti-HLA antibodies can form prior to transplantation in response to exposure to foreign antigens. There are a number of situations that place a child at risk of developing anti-HLA antibodies prior to transplantation, with some of these being common to all solid organ transplants and others being organ-specific. Common risk factors for the development of anti-HLA antibodies include the transfusion of blood products (especially those that contain leukocytes and platelets), previous organ transplantation, and a history of pregnancy (6,7). Risk factors that are unique to the cardiac population include previous cardiac surgery, especially surgery that requires exposure to homograft materials for surgical reconstruction, and the implantation of ventricular assist devices for mechanical support (6,8-12).

ANTIBODY TESTING

The presence and degree of anti-HLA antibody development is an important part of the pretransplant evaluation for a potential transplant candidate. HLA antibody screening is performed to determine the presence or absence of HLA antibodies and, with more recent testing, the HLA target and titers of these antibodies. Anti-HLA antibodies can be detected using HLA antigens that are either cell-based or part of a solid-phase assay (non-cell based) (3,13).

Cell-based strategies

Class I HLA molecules can be found on the intact cell membrane of either T- or B-lymphocytes, with Class II

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molecules being limited to B-lymphocytes. These cells provide the target antigens for the detection of anti-HLA antibodies in cell-based assays. Cell-based assays may rely on the binding of complement, as in the Complement-Dependent Cytotoxicity (CDC) assay, to determine the presence of HLA antibodies, or they can be conducted independent of the binding of complement, such as in flow cytometry (3). The CDC assay determines the percentage of lymphocytes that undergo cell death when a patient's serum is added in the presence of complement. Flow cytometry avoids the need for complement, as fluorescently tagged anti-human globulin is used to detect the presence of anti-HLA antibodies bound to the lymphocyte cell membrane. These tests can be used to determine the percentage of cell samples from a given population to which a recipient would react (panel reactive antibody) and in turn represent the HLA antigens that would be present in a donor pool from the same population (3). Both CDC assays and flow cytometry can also be used to determine whether a recipient has antibodies to a particular donor (crossmatch) and therefore help to predict the existence of a potential risk of antibody-mediated rejection if that organ is transplanted (3).

Cell-based assays range in sensitivity, with CDC methods being the least sensitive and flow cytometry being the most sensitive (2,3,14). These assays can result in both false positive and false negative results and do not allow for the determination of antibody specificity (2,3,15).

Solid-phase strategies

Recent developments have led to the creation of solid-phase assays. These assays utilize HLA antigens that are bound to a matrix and are not associated with a cell membrane. These solid-phase assays can be performed with soluble or recombinant HLA antigens bound to either plates (ELISA) or microbeads (flow cytometry or Luminex® multiplex platform) (3,13,16). These technologies have the advantage of not only determining the presence of HLA antibodies but also the class and specificity. A Panel Reactive Antibody (PRA) can be calculated using the known antigens in a donor pool and the antibodies detected by the solid-phase assay. Solid-phase assays are more sensitive than cell-based assays, with the level of sensitivity increasing from ELISA to flow-based technology. Unlike cell-based assays, these methods do not detect non-HLA antibodies and are unable to indicate whether an antibody has the capabilities of binding complement. However, these assays can detect anti-HLA antibodies below the threshold for a positive crossmatch (2,3,14,16). This level of sensitivity sometimes makes interpretation difficult because the clinical relevance of some of the antibodies that are detected remains unclear (3).

In clinical practice, the above information obtained pretransplant from the HLA lab is typically represented by the panel-reactive antibody (PRA) results. This number reflects the percentage of HLA antigens in a given donor population for which a recipient has antibodies. In general, a patient is considered to be sensitized if either the Class I or Class II PRA is $\geq$10%.

The HLA lab can also provide valuable information regarding recipient-donor matching at the time of potential donor evaluation, especially in the setting of living-related transplantation (e.g., kidney and liver), wherein a prospective crossmatch is performed during the assessment to determine the suitability of a particular donor-recipient pair. This technique requires the incubation of donor tissue with recipient serum to determine whether the antibodies bind. This can be performed by a CDC assay or flow cytometry. The biggest limitations to applying prospective crossmatching to deceased donor transplantation, for example, to heart transplantation, is the amount of time that it takes and the need for donor samples. An alternative option that addresses these issues is a virtual crossmatch. A virtual crossmatch is not a laboratory test but rather is a comparison of the known anti-HLA antibody specificities detected by solid-phase testing and the known donor HLA typing; using this approach makes it possible to avoid donors with HLA types that the recipient has developed antibodies to (3). Historically, the most common practice has been to perform a retrospective crossmatch. In this scenario, recipient serum and donor tissue are incubated after the transplant has occurred to determine whether there is evidence of antibodies against the donor. If the retrospective crossmatch is positive, most programs adopt different surveillance and management strategies to reduce the burden of antibodies and the risk of antibody-mediated rejection following transplantation.

THE CLINICAL RELEVANCE OF ANTI-HLA ANTIBODIES

Although debated in the past, it is now recognized that both class I and class II antibodies have an impact on outcomes posttransplantation. These antibodies have been associated with rejection following transplantation in many of the solid organs, including the kidney, liver, and lungs in adult populations (17-20), and have also been shown to be associated with decreased survival, graft loss, rejection, and vascular thrombosis following pediatric kidney transplantation (21-23).

The literature regarding the impact of preformed anti-HLA antibodies in pediatric heart transplantation remains limited. However, it has been well established in the adult heart transplant population that anti-HLA antibodies are associated with decreased survival and an increase in antibody-mediated cellular and chronic rejection (allograft vasculopathy) (24-29).

Currently, anywhere from 15-30% of pediatric patients listed for heart transplantation are sensitized (PRA$\geq$10%), with this number increasing in recent years (11,12,30). Earlier pediatric studies following heart transplantation have reported inconsistent results with respect to the role of elevated PRA on posttransplant outcomes. In a small cohort of pediatric heart transplant recipients, Jacobs et al. reported that an elevated CDC PRA did not affect 30-day survival (25% *vs.* 7.9%, $p=0.178$) but was associated with higher overall mortality (31). Wright et al. further explored this relationship by examining the impact of both an elevated PRA and the retrospective crossmatch results. The authors' analysis revealed no difference in graft survival between those patients who exhibited elevated PRA results versus those who were negative. However, when analyzed using crossmatch results, the authors clearly demonstrated that those patients with a positive retrospective crossmatch exhibited a worse overall survival ($p<0.015$) despite no difference in the time to cellular rejection, rejection grade, or the number of rejection episodes (32). In their analysis, the median time to graft loss was 15 months in those with a positive crossmatch, and no grafts with a positive crossmatch survived beyond 58 months. In contrast, a number of

smaller studies from other institutions reported that an elevated PRA did not affect survival posttransplantation (11,33).

Findings from the Pediatric Heart Transplant Study group, a large multi-institutional prospective database, concur with the findings of these smaller centers. These groups demonstrated that 6-month survival posttransplant was lower (77 *vs.* 93%) in those patients who exhibited a PRA≥50% compared with those with a PRA<10%. On further analysis, recipients with a PRA≥20% but a negative prospective crossmatch exhibited similar survival to those with a PRA<20%, but those with a PRA≥20% and a positive prospective crossmatch experienced a survival disadvantage at 1-year posttransplant. Interestingly, this study did not identify any difference in rejection outcomes or the development of vasculopathy based on sensitization status (12). In contrast, a single-center study has reported an association with elevated HLA antibodies and the later development of allograft vasculopathy (HR 2.76, CI 1.18-6.45, $p=0.019$) (11), and a more recent study has demonstrated no increased risk of antibody-mediated rejection in those with an elevated PRA but a negative prospective crossmatch (33).

The approach to patients with anti-HLA antibodies has varied over the years as research and understanding of this complex topic has expanded. Currently, many pediatric centers require a negative prospective or virtual crossmatch prior to accepting a donor organ to avoid the risk of antibody-mediated rejection and improve graft survival. However, this strategy itself has its own limitations (11,12,33,34). Feingold et al. (2007) have demonstrated, in their single-center study, that the mean time to transplantation was longer for those patients who required a negative prospective crossmatch (PRA >20%) and that there was a higher proportion of patients who died by 1 year after listing (22% *vs.* 8%, $p=0.055$) (11). Similar findings were reported in a larger multi-institutional study in which the one-year waitlist mortality was 19% for those with a PRA>50% compared with 9% for those patients with a PRA<10% (12). Furthermore, using the Organ Procurement and Transplant Network, the requirement for a prospective crossmatch at the time of listing was associated with longer waitlist times (248.7 ± 482.8 *vs.* 186.2 ± 504, $p<0.0001$), an increase in the waitlist mortality (HR 1.32, CI 1.10 to 1.56, $p=0.003$), and a decreased likelihood of achieving transplantation. In addition, this listing strategy was an independent predictor of waitlist mortality (HR 1.32, CI 1.10-1.56, $p=0.003$) (34).

Due to issues with the requirement of a negative prospective crossmatch, some centers have developed alternative strategies to decrease the antibody burden prior to or at the time of transplantation. This has been undertaken with the hope of improving posttransplant outcomes and decreasing the number of waitlist deaths.

PREVENTATIVE STRATEGIES

Prior to discussing strategies to address patients who are sensitized at the time of listing, it is important to discuss some strategies to prevent sensitization in patients who may be listed for transplantation or may require transplantation in the future. In patients with complex congenital heart disease, especially those with hypoplastic left heart syndrome, the avoidance of homografts or alterations of surgical materials to avoid antibody production may result in less sensitization (35). Because a number of these patients may require transplantation in the future due to a failing Fontan circulation, these precautions may decrease the risk of transplantation in this already high-risk, complex population. For those patients awaiting transplantation, avoiding exposure to HLA antigens is essential for the prevention of sensitization. This can include limiting platelet and plasma transfusions and using packed red blood cells that have been processed to remove leukocytes and platelets.

PRE- AND POST- TRANSPLANT MANAGEMENT

For patients who already exhibit preformed anti-HLA antibodies, there are a number of strategies that have been employed prior to or following transplantation to mitigate risk. The common goal of these strategies is to decrease the burden of the antibodies present. The targets for these strategies include removing preexisting antibodies and preventing the further production of antibodies. Although an exhaustive description of these strategies is beyond the scope of this chapter, some of the current strategies will be outlined.

Antibody removal can be achieved by plasma exchange in the operating room or by plasmapheresis pre- or posttransplant. Recently, protein-A immunoadsorption columns have also been used to decrease the circulating HLA antibodies without depleting all of the plasma components (36-40). These strategies are rarely used alone and often are combined with intravenous immunoglobulin (IVIG) and B-cell–directed therapies. Intravenous immunoglobulin (IVIG) has been used pretransplant for the purpose of desensitization and posttransplant in patients with a positive crossmatch. The mechanism by which IVIG works in this scenario remains unclear, but some proposed theories include the modulation of antibody and cytokine production, alteration of various signaling pathways, and complement inhibition (41,42).

Anti-B cell therapies have included the use of cyclophosphamide and mycophenolate mofetil, which deplete rapidly dividing cells and can result in decreased antibody production by inhibiting B-cell proliferation. Additional B-cell therapies include rituximab, which is a chimeric anti-CD20 monoclonal antibody that targets CD20-expressing cells. This antibody typically depletes CD20-expressing B-cells but does not deplete mature plasma cells that produce antibodies. Therefore, other agents that target plasma cells, including alemtuzumab (campath 1H) and bortezomib, have been proposed to fill this gap in the strategy to decrease the production of anti-HLA antibodies.

Desensitization, although common in kidney transplantation, has been utilized less in heart transplantation. The goal of desensitization is to decrease the HLA antibody load prior to transplantation to decrease the risk of rejection in the presence of antibodies. Various protocols have been used, predominantly in adult heart transplantation, with a combination of IVIG and rituximab with or without plasmapheresis (43-45). A few reports of desensitization pretransplant have also surfaced in heart transplant candidates with the use of immunoabsorption columns (36,37) and bortezomib (46). However, despite a limited number of reports, there is evidence from adult studies that a combination of plasmapheresis, IVIG, and rituximab

pretransplant can decrease the circulating antibody loads and increase the number of potential donors, with similar long-term survival and risk of allograft vasculopathy when compared with nonsensitized patients (45). In pediatrics, a similar approach using IVIG and rituximab was used in 14 patients with a PRA>10% without plasmapheresis. There was a significant reduction in the median calculated PRA in 8/14 patients. This strategy led to a median increase in the percentage of acceptable donors from 10% to 85%. Of the 8 responders, 5 were transplanted with one positive crossmatch and no detectable rejection (47). Long-term outcome data remain lacking.

Alternatively, some programs have opted to not desensitize patients prior to transplant but rather to address antibodies at the time of transplantation. Transplanting across a positive crossmatch must be weighed against waitlist mortality, posttransplant survival, and longer-term issues. Although this approach has been associated with increased risk, varying success rates have been reported in the pediatric heart transplant population. The limited number of pediatric donors and high waitlist mortality has primarily driven this approach. Holt et al. reported their experience with perioperative plasmapheresis, thymoglobulin, and cyclophosphamide in 17 patients with PRA>10% (48). Thirteen of these patients also exhibited a positive CDC crossmatch. Survival at 1 and 3 years was 85% and 73%, respectively, with these results being comparable to the reported outcomes from the ISHLT registry (48). The majority of these patients did experience early rejection; with many having both recurrent and hemodynamically significant episodes within the first 6 months posttransplant (48). The Hospital for Sick Children subsequently published their outcomes in transplanting sensitized patients. Their protocol included intraoperative plasma exchange, induction with thymoglobulin, posttransplant plasmapheresis (in those with a positive crossmatch), and a variety of B- and T-cell therapies depending on the clinical situation. During the study period from 1990-2006, 13 patients exhibited a PRA≥10%. Of these, 12 patients underwent plasmapheresis posttransplant for a positive donor-specific crossmatch. This cohort had a 3-month survival posttransplant of 89% with a 1-year survival of 71%. In the posttransplant period, 9 patients developed AMR, and 7 suffered ≥2R acute cellular rejection, with only 1 patient experiencing hemodynamic compromise due to rejection, which resulted in death. As seen in the previous studies, no patients developed AMR beyond 6 months posttransplantation (49).

Although these single-center studies have provided useful information, it remains unclear whether these findings can be improved upon or can be replicated in other centers. Currently, there is a multicenter National Institutes of Health Study examining a uniform strategy for treating sensitized patients in which the first available organ donor is accepted, regardless of the potential for a positive donor-specific crossmatch (50). This trial will provide further details about transplantation in this patient population and could provide a framework for improving outcomes in this ever-increasing population.

FUTURE DIRECTIONS

Although the techniques used to detect anti-HLA antibodies have improved, the significance and clinical relevance of the antibodies detected remain unclear. Recently, there have been many questions raised about the role of anti-HLA antibodies that are unable to bind complement, with the theory that the ability to bind complement may be one of the key factors in the determination of clinically relevant antibodies. Work at Stanford University has led to the development of an assay that can detect antibodies that bind the C1q component of the complement cascade (51). This assay has been used in the pediatric population and was found to detect a subset of patients at risk of developing AMR early after transplantation (51). This technique, though it requires further validation, does hold some promise for helping those practicing in the field of transplant to further understand the role of anti-HLA antibodies.

Sensitization is an issue that is increasing in frequency in the pediatric heart transplant population. Listing these patients for transplant with the requirement for a negative virtual or prospective crossmatch may decrease the risks posttransplant at the expense of increased mortality on the waitlist. Strategies to improve both the short-term and longer-term outcomes of those transplanted with a positive crossmatch are being explored with the hope of decreasing waitlist mortality and improving outcomes posttransplant in this patient population.

AUTHOR CONTRIBUTIONS

Conway J performed the research and wrote the manuscript, and Dipchand A edited the manuscript.

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