Transplantation in the Highly Sensitized Pediatric Patient

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The first cardiac transplant in the United States, and the second worldwide, was performed on a pediatric patient by Dr. Adrian Kantrowitz in the late 1960s. Acute cellular rejection and other factors led to poor outcomes, limiting the growth of pediatric and adult cardiac transplantation programs. Cyclosporine-based immunosuppression was introduced in the 1980s, and survival dramatically improved. A significant rise in annual cardiac transplantation volume ensued in the late 1980s but plateaued primarily because of a lack of donors. Use of long-term mechanical circulatory support devices (ventricular assist devices) in adults allowed the bridging of candidates to transplantation, combating long wait times resulting from a limited donor pool. These devices were often not suitable for smaller pediatric patients. Extracorporeal membrane oxygenation provides short-term pediatric support, but significant complications often develop, precluding transplantation. The Berlin Heart EXCOR Pediatric Ventricular Assist Device was developed for infants and small children in the early 1990s, gained approval for use in Europe in 1996, and was approved by the US Food and Drug Administration for use in the United States in 2011. Ventricular assist device use can lead to sensitization, another barrier to transplantation.

Sensitization, the development of circulating antibodies directed against human leukocyte antigen (HLA) proteins, poses a significant challenge. Risk factors for sensitization include exposure to blood products, infections, pregnancy, and use of ventricular assist devices. Surgery for congenital heart disease, especially with the use of human homograft tissue, is an additional risk factor for sensitization, affecting primarily pediatric patients. Although homografts are processed and preserved, they remain immunologically reactive and can lead to HLA antibody formation. These antibodies can be harmful to the transplanted graft. HLA antibody-mediated cardiac rejection (AMR), first described by Hammond and colleagues in the late 1980s, is a considerable risk factor for poor outcomes after pediatric transplantation. Listing or avoiding donor antigens before transplantation is but one strategy to mitigate early graft injury and loss resulting from HLA antibodies. Specifically, a transplantation program may decline an organ on the basis of the presence of antibodies that could recognize the donor antigens. This strategy, however, further constricts the donor pool and potentially prolongs the wait time.

The presence of HLA antibodies is thus a challenging problem before and after transplantation. This review addresses the following: (1) the role and classification of HLA antibodies, (2) detection methods to assess for antibodies and clinical implications, (3) therapeutic options, (4) clinical strategies toward the highly sensitized pretransplantation candidate, and (5) clinical strategies to combat AMR in the posttransplantation patient. Antibodies against major blood group (ABO) antigens represent another form of sensitization. This selected topic is addressed in a separate review as part of the series on Current Challenges in Pediatric Heart Failure and Transplantation.

Role and Classification of HLA Antibodies

Activation of progenitor B cells can lead to the development of mature B cells. This process occurs through either a T-cell–dependent or a T-cell–independent pathway. Once activated, the B cell matures into a plasma cell that manufactures antigen-specific antibodies. The role of antibodies is best known in vaccine strategies whereby exposure to the antigens of an inoculum lead to anti-infective protection (eg, measles vaccine). Human cells also express antigens, namely HLA antigens. Exposure to nonself human antigens can lead to HLA antibody formation. Unlike protective antibodies for infectious diseases, these HLA antibodies can be harmful. The transplanted organ expresses antigens unique to the genetic makeup of the donor. Immune recognition of donor antigens can lead to graft dysfunction through an antibody-mediated process. It is unclear whether all antibodies cause damage. Complement appears to play a key role in the antibody-mediated response. HLA antibodies can be classified as complement fixing antibodies (CFAs) and non–CFAs (Figure). It is widely accepted that only CFAs can activate the complement cascade through the classic pathway.

Detection Methods to Assess for HLA Antibodies and Clinical Implications

Numerous antibody detection methods have been available for decades. The initial tests were cell-based techniques. Patel and Terasaki developed the complement-dependent cytotoxicity (CDC) assay in the 1960s. This technique allowed the clinical detection of antibodies that were highly correlated with
posttransplantation graft failure. One limitation of the CDC assay is its inability to identify individual antibody specificities, especially in broadly sensitized patients. Additionally, it has been criticized for being highly variable, subjective, undersensitive, and for lacking standardization. Solid-phase techniques were developed to overcome these limitations. The single antigen bead (IgG-SAB) assay in particular has revolutionized the field, sensitively and specifically identifying anti-HLA IgG antibodies. Despite high test sensitivity, the detected antibodies do not always correlate with clinical outcome. Unlike the CDC assay, most solid-phase assays detect the whole gamut of circulating antibodies and do not discern between CFAs and non-CFAs. Despite this limitation, solid-phase assays have identified the presence of significant antibodies in subjects indicated to be nonsensitized by the CDC assay. The C1q-SAB assay was developed at Stanford University to overcome the limitations of current solid-phase assays. This assay detects only CFAs, and the resultant number of antibodies identified typically is lower compared with the other tests. The importance of this highlight is of the concept of the panel-reactive antibodies (PRA). The PRA estimates the percentage of incompatible donors a candidate might encounter as a result of unacceptable antibody-antigen interactions. For example, if a candidate has circulating HLA-A2 antibody, the PRA would be 48% in the United States. If considered a significant antibody, 48% of potential donors would be unacceptable because of the frequency of this antigen in the population. Thus, the clinical challenge is how best to assess for problematic antibodies. Assays that do not distinguish between CFAs and non-CFAs could falsely identify a larger number of unacceptable antigens and further limit the donor pool. The C1q-SAB assay thus could play an important role in these circumstances.

Although the C1q-SAB assay might reduce the PRA by removing the antibodies thought not to be clinically harmful at the time of transplantation, others have used mean fluorescence intensity (MFI) for risk stratification. Antibodies detected by solid-phase assays are expressed as the MFI, representing the strength of antibody binding. Commonly, an MFI >8000 is considered strong binding, an MFI of 2000 to 8000 is considered moderate binding, and an MFI of 1000 to 2000 is considered weak binding. Notably, these assignments are determined by individual laboratories, and consensus on thresholds is lacking. Despite this limitation, there are reports correlating higher MFI with positive T- and B-cell cross-matches. A cross-match functionally determines whether antibodies can lead to donor cell death. Rather than focusing on MFI, Zeevi and colleagues investigated the association of antibody titer and the ability to bind complement. They found poor correlation in undiluted sera between antibody detected by the IgG-SAB assay and presence of CFAs. When the samples were diluted, however, the correlation between the IgG-SAB assay and the undiluted C1q-SAB assay was significantly enhanced.

The practical goal of identifying clinically important circulating HLA antibodies is to avoid allograft rejection and graft loss. Patients are at risk because of either preexisting antibodies present before transplantation or de novo antibodies developing after transplantation. Large cohorts of pediatric patients in prospective and retrospective studies have shown worse outcomes among those with PRA values >10%. Survival after pediatric heart transplantation is particularly inferior for sensitized patients with preformed donor specific antibodies (DSAs) at the time of transplantation and a positive cross-match. The Arkansas Children’s Hospital reported 14 patients who underwent transplantation across a positive cross-match. No difference in 1-year survival was noted compared with a cross-match–negative cohort, but significantly worse outcome was seen by 2 years, with no graft surviving beyond 58 months. The importance of discerning between CFAs and non-CFAs is highlighted by the Columbia University pediatric study, which used the CDC and solid-phase assays together to identify patients who might benefit from enhanced antibody-depletion therapies. Significantly worse outcomes were noted on the basis of the presence of CFAs. This laboratory also found that IgG-SAB MFI values did not accurately predict which antibodies would bind complement, even at values exceeding 10000 MFI. The C1q-SAB assay from Stanford University was used clinically in a pediatric heart transplantation cohort. Preexisting C1q-SAB–positive DSAs correlated highly with a positive retrospective cytotoxic cross-match. Whereas presence of non-CFAs alone was not detrimental, the addition of CFAs in circulation was highly predictive of AMR early after transplantation. Researchers at the University of Pittsburgh Medical Center came to the same conclusion that the presence of DSAs detected by the C1q-SAB assay after transplantation significantly correlated with the development of AMR. They also demonstrated the importance of epitope-specific reactivity to further identify harmful antibodies. With the use of the HLAMatchmaker computer algorithm, a specific HLA-A2 epitope was identified that caused cytotoxicity in a pediatric heart transplant recipient. Although this system requires high-resolution HLA typing that is not widely available, epitope-specific typing and matching may eventually help identify concerning antibodies and broaden the limited donor pool.

**Overview of Therapeutic Options**

There is neither a universally accepted method for achieving desensitization nor standard methods for measuring the efficacy of the techniques used to achieve that goal. A survey of 23 cardiac transplantation centers worldwide found that some combination of at least 9 different medications and therapies was used for the purpose of desensitization, with the threshold PRA for the initiation of therapy varying greatly by center. The bulk of therapies involved plasmapheresis, intravenous immunoglobulin (IVIG), and anti–B-cell agents.

Plasmapheresis and immunoabsorption remove antibodies from circulation. Plasmapheresis separates plasma from

![Figure](image-url) The total antibody burden present in a sensitized patient is composed of non–complement fixing antibodies (Non-CFAs) and complement fixing antibodies (CFAs).
the cellular components in blood, indiscriminately removing proteins, including HLA antibodies. Unfortunately, antibody levels often rebound shortly after treatment. Alternatively, immunoadsorption passes serum over a column containing beads bound to a factor that binds antibodies. These modalities are often used in combination with other immunomodulatory therapies, particularly IVIG. The mechanisms of action of IVIG are not clearly understood, but popular theories include the induction of anti-idiotypic antibodies, the inhibition of cytokine gene activation, T-cell receptor antagonism, the disruption of antigen presentation, anti-CD4 properties, cytokine receptor agonism, and the inhibition of the membrane attack complex.

Therapies aimed at reducing antibody production typically target the B cell. Rituximab, a monoclonal antibody to the B-cell marker CD20, works through reduction of preplasma cells via depletion of B lymphocytes in the circulation, lymph nodes, and bone marrow. Cyclophosphamide interferes with rapidly dividing cells and is known to selectively inhibit the proliferation of B cells.

Although plasmapheresis removes antibodies from circulation and IVIG and rituximab deplete B-cell populations, they do not have a direct or significant effect on the antibody-producing plasma cell. Bortezomib, a proteasome inhibitor used for the treatment of the plasma cell cancer multiple myeloma, shows potential in treating highly sensitized patients. Proteasome inhibitors function through interference of the cell cycle, causing accumulation of cell cycle proteins and ultimately lysis of the plasma cell. The action of these inhibitors may not be confined to depleting plasma cell populations and appears to limit naïve and memory B-cell proliferation.

**Clinical Strategies for the Highly Sensitized Pretransplantation Candidate**

Elevated PRAs in pediatric patients are associated with greater wait-list mortality as a result of the prolonged time to find a suitable match. Strategically, a reduction of circulating antibodies is one approach to improve the chances of successful transplantation. Although plasmapheresis monotherapy and IVIG monotherapy have been described, most of the published desensitization protocols use a multipronged approach and have focused on adult populations. Pediatric literature on pre–heart transplantation desensitization is limited to case reports and small series. Balfour and colleagues published the case report of a highly sensitized child who had developed broad reactivity after multiple antigenic exposures, including surgical placement of human homografts for cardiac palliation. Initially, IVIG and mycophenolate mofetil (MMF) were used without effect on the PRA. Plasmapheresis temporarily lowered the number of antibodies, but they quickly rebounded. Ultimately, rituximab was used, lowering the PRA to 18%. In a single-center prospective study, Schumacher and colleagues enrolled 14 pediatric patients with PRA >10%. Patients were given high-dose IVIG and rituximab, with repeat doses if CD20 counts exceeded 10 to 20 cells per 1 mL. Eight patients were responders, with 6 requiring 2 to 3 cycles for significant reduction of the PRA. This treatment paradigm increased the donor pool for the patients, with no rejection after transplantation in the responders.

Little is reported about bortezomib for pre–cardiac transplantation desensitization. To the best of our knowledge, only 1 study from Patel and colleagues described the use of bortezomib and plasmapheresis in 7 adult patients awaiting transplantation. The mean PRA dropped from 62% to 35% after a schedule consisting of 4 cycles of plasmapheresis with bortezomib.

Despite attempts to achieve pretransplantation desensitization, PRA may remain elevated, and the likelihood of a negative cross-match remains low. Transplantation under these circumstances may result in hyperacute rejection shortly after implantation, characterized by immediate graft dysfunction and recipient death. Alternatively, transplantation may not be considered an option. Rather than exclude highly sensitized candidates, some pediatric centers have reported strategies to manage the antibody-antigen response during the peritransplantation period. Jacobs and colleagues reported 8 children with elevated pretransplantation PRA (>10%) who received IVIG weekly and either cyclophosphamide or MMF. Plasmapheresis or exchange transfusions were used at the time of implantation and postoperatively for up to 5 days. Standard posttransplantation immunosuppression was modified, starting with cyclophosphamide with eventual conversion to MMF. Short-term mortality, however, was inferior compared with the nonsensitized group.

The pediatric group in Toronto, ON, Canada, described its experience with 13 sensitized children using perioperative plasmapheresis and thymoglobulin induction. Administration of rituximab just before graft implantation was added to the protocol later in their series. Plasmapheresis was continued postoperatively when the retrospective cross-match was positive. Anti–B-cell therapy consisted of cyclophosphamide in the first 2 patients, later replaced by rituximab if there was evidence of AMR, graft dysfunction, or an increase in antibody titers or B-cell count. Despite the presence of AMR in 9 patients, only 1 patient had hemodynamic compromise and died, and no patient developed AMR beyond 6 months after transplantation. One-year survival in this cohort was 71% compared with 84% in a nonsensitized population.

The St. Louis pediatric group reported its experience aggressively targeting the T-cell pathway. Seventeen sensitized patients (PRA >10%) were enrolled in that protocol for transplantation without a prospective cross-match. Plasmapheresis was used before transplantation, and afterward, a retrospective cross-match was performed. If positive, plasmapheresis was continued for 5 to 7 days. T-cell cytolytic therapy (ATGAM or thymoglobulin) was used for induction and continued for 7 to 14 days if the cross-match was positive. Anti–B-cell therapy consisted of cyclophosphamide given for 4 weeks and then replaced with azathioprine or MMF. Thirteen of the 17 sensitized patients underwent transplantation against a positive CDC cross-match. Rejection was seen frequently, with most events occurring within the first posttransplantation month. Seven episodes of rejection were associated with hemodynamic compromise, with 1 secondary to hyperacute rejection and 3 with high-grade cellular rejection. One- and 3-year survival rates were 85% and 73%, respectively. Although outcomes were not statistically compared with a nonsensitized cohort, this study demonstrates that very-high-risk patients can
successfully undergo transplantation across a positive CDC cross-match with reasonable short-term survival outcomes.

The pediatric group at Boston Children’s Hospital applied a standard peritransplantation antibody-depletion protocol to sensitized patients. Sensitization was defined as a PRA >10%, and 33 patients underwent intraoperative plasmapheresis or plasma exchange before cardiopulmonary bypass. A CDC cross-match was performed in conjunction with transplantation, and if it was positive, plasmapheresis was continued in the ensuing postoperative days (n=12). Scheduled doses of IVIG were also given postoperatively. If the CDC cross-match was negative, the patient did not receive further antibody-depletion therapy beyond the operating room. Survival outcome, time to first rejection, and number of rejection episodes in year 1 were statistically similar between CDC-positive and CDC-negative patients. Hemodynamically compromising AMR in the first year, however, was seen more commonly in the CDC-positive patients (50%). Treated infections were also more common in this cohort. Regardless, this study also demonstrates that excellent comparative survival outcomes can be achieved in this very-high-risk population.

**Clinical Strategies to Combat AMR in the Posttransplantation Patient**

Management of sensitized patients beyond the initial postoperative days is directed at preventing the acute and chronic effects of the antibody-mediated response. Sensitized patients appear to be at increased risk for AMR and coronary artery vasculopathy (CAV). Although neither presents as dramatically as hyperacute rejection, both have significant effects on post-transplantation morbidity and mortality. Treatment of AMR beyond the first few postoperative days may include ongoing plasmapheresis to achieve antibody clearance. Serial monitoring for DSAs helps guide clinical decisions, including length of therapy. AMR with graft dysfunction in the first few weeks after transplantation often requires escalation of immunomodulatory therapies, including the addition of rituximab to limit future plasma cell production or replacement of MMF with cyclophosphamide. Although there is limited evidence for use in heart transplantation, proteasome inhibitors have demonstrated efficacy in treating AMR in kidney transplantation patients. Everly and colleagues and Woodle and coworkers have developed the Cincinnati proteasome inhibitor–based regimen, including cycles of plasmapheresis, rituximab, and bortezomib. Although bortezomib significantly reduces all class I DSAs, it is less effective at removing class II antibodies. Repeated cycles of proteasome inhibitor therapy may be required to achieve a reduction in targeted antibodies. Use in pediatrics has been limited. The Arkansas Children’s Hospital group reported 5 children unsuccessfully treated for rejection with multiple rounds of plasmapheresis, IVIG, and rituximab. Despite therapy, DSAs persisted coexistent with AMR and hemodynamic compromise. Bortezomib therapy immediately reduced circulating DSAs with a significant improvement in cardiac function and resolution of biopsy-evident AMR.

Eculizumab is a humanized monoclonal complement inhibitor that offers another approach to the treatment of AMR in the setting of graft dysfunction. It targets a terminal complement product preventing complement activation and formation of membrane attack complex, the terminal event that “punches holes” in the target cell (donor cardiomyocytes). Interference of membrane attack complex formation could limit the effect of antibody action in AMR. To the best of our knowledge, only anecdotal cases of eculizumab use in heart transplantation exist. Successful use has been reported in the renal transplantation literature either alone or in conjunction with other traditional therapies aimed at antibody reduction. Longe and colleagues also reported the combination of eculizumab and bortezomib therapy in the setting of AMR after renal transplantation. A strategy to prevent AMR in the first 3 months after transplantation in a high-risk renal transplantation cohort was used by Stegall and colleagues in one of the largest open-labeled eculizumab studies to date. Twenty-six patients received eculizumab compared with a control group of 51 patients. No differences were seen in percent of patients who developed high levels of DSAs. All those in the control group who developed DSAs had AMR compared with 15% of patients in the treatment group.

The need to treat for the presence of antibody in the absence of AMR is unclear because B-cell therapies are typically used in the setting of rejection with graft dysfunction. Arguments for prospective intervention include the risks associated with circulating antibody. For example, independent risk factors for the development of CAV include the development of de novo class II antibody and asymptomatic AMR. Lietz and colleagues reported that de novo isotype switch and antibody type, specifically IgG HLA class II antibody, may be important determinants of CAV risk. Irving and colleagues reported 59 pediatric patients, 3 of whom developed de novo class II DSAs and either died or required retransplantation as a result of complications related to CAV. Xydias and colleagues further support the premise that the presence of HLA class II antibodies after pediatric transplantation correlates with the development of CAV.

The appropriate timing of AMR assessment and surveillance has been debated for many years. Recently, an International Society for Heart and Lung Transplantation consensus group developed guidelines for the routine assessment of protocol biopsies for AMR. Current recommendations include scheduled assessment of protocol biopsies for AMR by immunostaining and assessment of AMR by immunostaining when clinically suspected.

**Summary**

Sensitization against HLA antigens is a growing problem in the field of pediatric cardiac transplantation. Although surgical outcomes for congenital heart disease have improved over the decades, these successes have added to the growing list of sensitized patients who eventually may require transplantation. Cardiac transplantation survival has improved, but morbidity and mortality secondary to HLA antibodies hinder outcome. Aside from acute hemodynamic compromise, there is compelling evidence linking sensitization and AMR with the development of CAV, a major limiting factor affecting long-term graft survival. Clinical advances have improved our understanding of the roles of antibody type, CFAs and non-CFAs, and DSAs and non-DSAs. Therapeutic strategies
target both the T- and B-cell lines. Combinations that include plasmapheresis, IVIG, cyclophosphamide, and rituximab have been used in clinical studies with variable success. Two newer agents show promise, targeting both ends of the antibody-mediated spectrum: Bortezomib depletes plasma cell populations, and eculizumab blocks the terminal effects of antibody action, thus preventing myocardial cell dysfunction and death. Despite numerous diagnostic and therapeutic advances, many questions remain about the best approaches. The role of HLA antibodies remains the central target of investigation.

Disclosures

None.

References


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