Pediatric Heart Transplantation in Human Leukocyte Antigen–Sensitized Patients

Evolving Management and Assessment of Intermediate-Term Outcomes in a High-Risk Population

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Background—There is an elevated risk for poor outcomes after heart transplant (HTx) in patients sensitized to human leukocyte antigens including graft dysfunction, acute cellular and antibody-mediated (AMR) rejection, and cardiac allograft vasculopathy. We report our experience with human leukocyte antigens–sensitized pediatric HTx recipients.

Methods and Results—We identified pediatric HTx patients with elevated pre-HTx Panel Reactive Antibody (Class I/II; >10%), or a positive T- or B-cell crossmatch. Thirteen patients met criteria (5 female, 39%). The median age at HTx was 7 months (3.5 months to 15.5 years). Nine were infants who had prior palliation for congenital heart disease. Four were older patients (median 7.3 years; 4.8 to 15.5 years): 2 had congenital heart disease (Fontan), 2 were re-HTx. B-cell therapies were used in all patients, guided by assessment of CD19+ and CD20+ cells. Immunosuppression included thymoglobulin induction, and tacrolimus, mycophenolate mofetil, and steroids. Daily plasmapheresis ± intravenous immunoglobulin G was used if there was a positive crossmatch on day 1, with a gradual, biopsy-guided weaning schedule. Rituximab was used when AMR was detected on biopsy: more recently (n = 3), used empirically peroperatively. AMR was confirmed in 9 patients within median 0.9 months post-HTx. Seven had early acute cellular rejection (≥ ISHLT Grade 2 R) with no hemodynamic compromise or graft dysfunction. There were 4 deaths post-HTx (range, 11 days to 9 months). The median follow-up of 9 survivors was 1.7 years (0.3 to 3.7 years). Of 7 patients >6 months post-HTx, no AMR or cardiac allograft vasculopathy was observed at a mean of 1.9+1.1 years post-HTx and no cardiac allograft vasculopathy.

Conclusions—Despite aggressive management, acute cellular rejection and AMR occurred frequently early post-HTx. An algorithm of B cell–directed strategies can be effective in managing these patients with reasonable intermediate-term outcomes. (Circulation. 2007;116[suppl I]:I-172–I-178.)

Key Words: pediatric heart transplantation HLA-antibodies sensitization outcomes

There is an elevated risk for deleterious outcomes after heart transplantation (HTx) in patients sensitized to human leukocyte antigens (HLA) including graft dysfunction, histological damage, allograft rejection (acute cellular [ACR] and antibody-mediated [AMR]), and chronic rejection/cardi-.allograft vasculopathy (CAV). 1–8 There has been an increase in heart transplant candidates allosensitized to HLA antigens over the years 9 which may result from exposure to blood products, 5,10 homograft material used in surgical palliation of congenital heart disease, 11,12 use of ventricular assist and mechanical support devices, 4 and in a growing number of patients requiring retransplantation. 7

Determinations of Panel Reactive Antibody (PRA) are done to delineate a patient’s potential for sensitization to donor HLA antigens. 13 Antibodies to HLA are assessed using lymphocytotoxic assays of preformed reactive antibodies 14 and more recently using solid-phase assays with microbeads coated with purified HLA antigens. 15 Patients with a reaction to >10% of antigens (to either Class I or II) are generally considered to be allosensitized. 13,14,16 However, this is allosensitivity to a ‘random’ donor; having a positive crossmatch with the actual donor (eg, HLA antibody toward donor alloantigens) at the time of transplant has been clearly demonstrated to increase the risk for poor outcome after transplant. 14,17 Donor-directed HLA antibodies increase the risk for post-transplant complications in the cardiac allograft. 1,6 Both antibodies to Class I and Class II antigens appear to be related to an elevated risk of rejection. 18 ACR,
AMR, and CAV are all associated with a donor specific positive cross-match.4–6,13 In particular, anti-Class II antibodies have been linked to an increased incidence of rejection, CAV, and rejection-related mortality.2,19 Furthermore, patients with PRA >10% pretransplant and a positive cross match are at high risk for graft loss.4,9,20 Lavree and colleagues demonstrated that in addition to an increased risk for hyperacute rejection, there was a direct relationship between elevated PRA and ACR in the first 3 months post-transplant, which in turn increased the risk of CAV.20

Allosensitized patients may be excluded from HTx, restricted to certain donors, or experience prolonged waiting times.35 Some transplant programs require a prospective negative donor-specific cross-match for patients with PRA screens >10% to 15%,14,21 some exclude highly sensitized recipients, and a few centers use strategies to attempt to reduce the formation of circulating cytotoxic alloantibodies before transplantation (desensitization) using treatments (alone or in combination) such as intravenous immune globulin (IVIG), cyclophosphamide, mycophenolate mofetil (MMF), and rituximab.1,5,14,22–24 Although prospective cross-matching has not routinely been done for consideration of donor acceptance at our institution, we have adopted an evolving aggressive pre- and perioperative management protocol that includes as possible options: pretreatment of sensitized patients, intraoperative plasma exchange, post-transplant plasmapheresis, and T- and B-cell suppression rather than excluding such patients from HTx. We evaluated our experience with HLA-sensitized pediatric HTx recipients in order to report our management strategies and assess intermediate-term outcomes.

Methods
After institutional research ethics board approval we retrospectively reviewed the records of all HTx recipients at the Hospital for Sick Children from 1990 to May 2006. Patients with elevated pretransplant PRA (Class I or Class II; >10%), or a positive T- or B-cell crossmatch were identified. Detailed information about medical history were collected including age at HTx, gender, diagnosis, previous cardiac and other surgery, blood exposure, use of extracorporeal mechanical oxygenation, mechanical support, or ventricular assist device, wait-time for HTx, pretransplant PRA level, antibody specificity, donor and recipient serotype or molecular HLA typing, and donor-specific cross-match results.

Preoperative treatments, perioperative and post-transplant management including immunsuppression, plasmapheresis, and T- and B-cell strategies used are described. Outcome measures include graft and patient survival and are depicted by the actuarial survival method described by Kaplan and Meier. Rejection (ACR and AMR) was reported descriptively and defined by endomyocardial biopsy-proven rejection using International Society for Heart and Lung Transplantation (ISHLT) nomenclature.25 For ACR, moderate rejection (≥ISHLT Grade 2 R) was reported. AMR I reflects presence of AMR and includes any of the following characteristics: positive immunofluorescence staining for C4d, C3, fibrinogen; deposition of IgG, or histological features of AMR.25 All biopsies in sensitized patients with a positive donor-specific crossmatch undergo routine immunofluorescence on a frozen sample for AMR. CAV was defined (by institutional protocol) either by dobutamine stress echocardiogram (DSE) (performed annually starting at 6 months post-Tx) or angiographically (performed annually over the study period starting at 1 year post-Tx in all patients ≥10 kg). CAV was reported as elapsed time between HTx and first documentation of CAV.

Incidence of rejection was compared for patients who received pretreatment therapy compared with those who did not using χ² frequency analysis. All data analyses were conducted using SAS statistical software (SAS Institute Inc), with a probability value of <0.05 considered as statistically significant. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Pretransplant PRA/HLA Determination
Before 2003 PRA was assessed in all patients using a Complement-dependent Cytotoxicity Anti-human Globulin (CDC-AHG) cytotoxic assay on a panel of fresh T cells covering the spectrum of HLA types in the local donor population (only patient 1 was identified as sensitized using this method). All other patients (n=12) in this cohort (since January 2003) had PRA determination by solid phase flow beads (Tepnel Lifecodes—using Lifematch Fluoroanalyser [Luminex] reagents). The solid phase flow bead assay is both IgG and anti-HLA specific; whereas, the CDC-AHG assay is neither IgG nor HLA specific. It is universally accepted that anti-HLA alloreactive antibodies are more clinically relevant than other antibodies, making this latter assay more specific to the transplantation population.

Patients were considered positive for anti-HLA antibodies when they had a PRA ≥10% (either Class I or II). Any patient that exhibited alloreactivity was then further assessed as to HLA specificity of either the Class I or Class II antibodies. In one patient identified as allosensitized (before 2003) antibody specificity was determined using HLA specificity ELISA plates (Quik-ID either Class I or II from GTI Inc). Subsequently, all specificity assays were done using the Tepnel Lifematch specificity fluoroanalyzer beads (Tepnel Lifecodes).

Results
Patient Characteristics
Over the study period, there were 183 heart transplants in 167 patients. Of these, 13 patients (8%) met criteria for being “allosensitized” before HTx (see Methods). The median age at HTx was 7 months (range: 3.5 months to 15.5 years). Nine were infants (<12 months), 6 of whom also received ABO-incompatible grafts.26 All infants had prior surgical palliation (staged Norwood). There were 4 older patients transplanted at a median age of 7.3 years with the following diagnoses: failed Fontan,2 and retransplant,2 (original diagnoses were cardiomyopathy and failed Fontan; Table 1).

Overall Survival
There were 4 deaths ranging from 11 days to 9 months post-HTx (Table 2). The median follow-up time of the 9 survivors was 1.7 years (range, 3 months to 3.7 years). Three month and 1 year actuarial survival of this cohort was 89 and 71%, respectively (Figure).

Pretransplant Immunology
The profile of patients’ immunologic status before HTx is presented in Table 3. Table 4 depicts patients’ specific antibody status and donor specific antibodies to Class I and II HLA antigens in the immediate period preceding transplant (0 to 4 weeks; results are those closest to transplant).

Pretransplant Treatment to Reduce PRA
Five patients between 2002 to 2004 received treatment with weekly IVIG (1 g/kg) for varying periods of time or oral low dose MMF (20 mg/kg/d) before transplant in an attempt to reduce circulating alloantibodies (Table 5). Post-treatment antibody results suggest that intervening treatment may have
had an impact on reducing antibody quantity, but not necessarily breadth (thus, although percentage PRA might not have changed much, the mean antibody quantity was reduced). However concern for rebound B-cell antibody production remained and patients who were pretreated also received post-transplant therapy to halt antibody production.

Perioperative Management

Plasma exchange was done on cardiopulmonary bypass in all patients as described previously.²⁶ Splenectomy was not used. All patients received perioperative induction with thymoglobulin as per institutional protocol.

In the most recent 3 sensitized patients (since January 2006), given positive clinical experience with rituximab post-transplant, our protocol was modified so that rituximab (one dose) was given empirically perioperatively (either on call to the operating room or during the operative procedure). Rituximab is an anti-CD20 monoclonal antibody that rapidly causes destruction of CD20 positive cells—primarily B cells and their precursors.²³,²⁷ Plasma cells, which are important for the production of antibodies, are not affected.²³,²⁷

Post-Transplant Management

Induction therapy with thymoglobulin (1.5 mg/kg/d) was used for 2 to 7 days. Immunosuppression included standard triple immunosuppression with tacrolimus, MMF, and steroids as per institutional practice. Two patients received sirolimus, both for renal sparing purposes. Patients underwent early endomyocardial biopsy-proven (within 2 weeks post-HTx) with immunofluorescence staining for IgG, IgA, IgM, C4d, C3 and fibrinogen.

All donor-specific crossmatches were done retrospectively with results available within 24 hours post-HTx. Plasmapheresis was instituted in the face of a positive donor-specific crossmatch in 12 patients daily for 5 days followed by a gradual, biopsy-guided weaning schedule (range 1 to 24 weeks; Table 6). Titers of donor-specific antibodies were assessed at the time of plasmapheresis and each biopsy and used for individualization of plasmapheresis plan. Plasmapheresis was weaned and eventually discontinued when there was no further evidence of AMR by histology. There was no specific level of donor-specific antibodies that lead to a decision to discontinue plasmapheresis.

### TABLE 1. Patient Characteristics (n=13)

<table>
<thead>
<tr>
<th>Factor</th>
<th>n (%)</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median wait-time (days)</td>
<td></td>
<td>32 (4–126 days)</td>
</tr>
<tr>
<td>Age at transplant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12 mos</td>
<td>9</td>
<td>7 months (3.5–8 mo)</td>
</tr>
<tr>
<td>≥12 mos</td>
<td>4</td>
<td>7.3 years (4.8–15.5 years)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>5 (38)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8 (62)</td>
<td></td>
</tr>
<tr>
<td>Transplant status (Canadian)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Status 1</td>
<td>2 (153)</td>
<td></td>
</tr>
<tr>
<td>Status 2</td>
<td>1 (8)</td>
<td></td>
</tr>
<tr>
<td>Status 3/3.5</td>
<td>7 (54)</td>
<td></td>
</tr>
<tr>
<td>Status 4</td>
<td>3 (23)</td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>De novo HTx</td>
<td>11 (85)</td>
<td></td>
</tr>
<tr>
<td>CHD</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Norwood</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Fontan</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Retransplant</td>
<td>2 (15) (1 failed Fontan, 1 CM)</td>
<td></td>
</tr>
<tr>
<td>ABO-incompatible graft</td>
<td>6 (46)</td>
<td></td>
</tr>
</tbody>
</table>

CM indicates cardiomyopathy; CHD, congenital heart disease

*Status 1,2,3—UNOS equivalent Status 2. Status 3.5,4—UNOS equivalent Status 1

### TABLE 2. Mortality: Time and Cause of Death

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time of Death Post-Transplant</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>9 months</td>
<td>Infection, MOF</td>
</tr>
<tr>
<td>11</td>
<td>3 months</td>
<td>Aspiration</td>
</tr>
<tr>
<td>12</td>
<td>27 days post-HTx #2</td>
<td>Sudden unexplained death during plasmapheresis Autopsy: mild ACR (1 R), no AMR, no CAV</td>
</tr>
<tr>
<td>13</td>
<td>11 days</td>
<td>Rejection: Severe ACR and AMR (on ECMO support)</td>
</tr>
</tbody>
</table>

MOF indicates multisystem organ failure.
Attempts to target B cells were used in all patients. Cyclophosphamide was used early in our experience in 2 patients (2002/2004). More recently, rituximab (see above) was used post-HTx in 9 patients if (1) there was AMR detected on biopsy, or (2) there was evidence of graft dysfunction on echocardiogram, or (3) there was an increase in antibody titers or B-cell count (CD20) in the face of evidence of ongoing AMR. Fluorescent activated cell sorter/flow cytometry assessment of lymphocyte subsets was performed to follow CD19+ and CD20+ B-cell counts in patients pre- and post-rituximab.

Intermediate-Term Outcomes

Table 6 summarizes the post-transplant outcomes for intensive care unit stay, rejection, infection, CAV and post-transplant lymphoproliferative disease (PTLD). Although the median intensive care unit stay was relatively short, 2 infant patients experienced difficult post-transplant courses attributable to multifactorial and multisystemic reasons (chronic lung disease, paralyzed diaphragms, recurrent infections, gastrointestinal problems, etc) leading to prolonged stay in intensive care unit over 3 months with 1 death.

Rejection

There was histological evidence of AMR confirmed in 9 patients at a median of 0.75 months post-HTx (range: 1 to 4 weeks). In addition, 7 patients had moderate biopsy-confirmed ACR (ISHLT former Grade 3 A or revised ISHLT Grade 2 R; Table 6). No patient experienced any hemodynamic compromise or compromise to systolic function by echocardiography related to any of these episodes of rejection with the exception of Patient 11 who died.

Of 7 patients >6 months post-Tx at the time of data analysis, duration of plasmapheresis treatment ranged from 2 to 5 months post-transplant. The timing of resumption of antibody production varied between patients after the first dose of rituximab. Patients were followed by fluorescent activated cell sorter/flow cytometry analysis of CD19+ and CD20+ count and quantitative titers of donor-specific anti-HLA antibodies at the time of each biopsy. Five patients received a second dose of rituximab for ongoing management of AMR based on timing to resumption of documented B-cell recovery, anti-HLA antibody production, and the presence of any ongoing AMR by biopsy criteria.

No patient developed AMR on follow-up beyond 6 months post-Tx (median follow-up 1.7 years, range 0.3 to 3.7 years; Table 6), despite documented return of donor-specific anti-HLA antibodies in 6 patients. One patient had positive immunofluorescence on biopsy (weak C3 and C4d; trace IgM) at 2 years post-HTx with no histological evidence of AMR and normal graft function. The clinical significance of this is unclear, but the patient is being followed expectantly.

In this patient cohort, there was no statistical difference in incidence of humoral rejection in patients who received pretreatment with IVIG/MMF before HTx.

Graft Vasculopathy

None of the 9 survivors have been diagnosed with CAV to date at a median follow-up of 1.7 years (range 0.4 to 3.7 years). One patient has an incidental finding of small vessel intimal hyperplasia on endomyocardial biopsy-proven at 2 years post-HTx with no histological evidence of AMR and normal graft function. The clinical significance of this is unclear, but the patient is being followed expectantly.

In this patient cohort, there was no statistical difference in incidence of humoral rejection in patients who received pretreatment with IVIG/MMF before HTx.
never been discharged from hospital) was found on autopsy to have mild CAV that was not clinically apparent (Table 6).

Infections and PTLD
There were no cases of PTLD or malignancy in this cohort. Infectious complications early post-HTx (before hospital discharge or death) occurred in 5 patients, most commonly respiratory or central-line related inserted lines (Table 7). The incidence was no different than our nonsensitized transplant or nontransplant cardiac surgery population.

Discussion
Although sensitized pediatric HTx recipients have historically been considered to be at high risk for a poor outcome, our results demonstrate that reasonable intermediate term outcomes are feasible. Similar to other studies, we found that sensitized children who underwent HTx frequently developed AMR early post-HTx. However, with early aggressive intervention, no survivors developed AMR beyond 6 months post-HTx.

Overall Survival
Jacobs and colleagues reported significantly greater mortality for sensitized than nonsensitized pediatric heart recipients (50% versus 15.4%, \( P = 0.043 \)). This observation is found elsewhere in the literature, both in adult and pediatric patients. In our experience, sensitized patients (4/13) experienced an actuarial survival of 89% and 71% at 30 days and at 1 year which is lower than our overall institutional actuarial survival (30 day and 1 year survival of 95% and 84%, respectively) but very encouraging in this “high risk” population.

Pretreatment
There is literature indicating that preTx IVIG may help to decrease PRA in some, but not all patients. MMF has also been used to decrease PRA. Our attempts to reduce circulating antibodies in 5 patients, though successful from a quantitative perspective (Table 5), have not reduced the occurrence of rejection after transplant. It is noteworthy that the PRA is a measurement of the breadth and not the depth of antibody. The use of plasmapheresis and IVIG likely impacted on the depth without affecting breadth (hence, although some patients did not have a large change in percentage PRA, that does not mean antibody quantity was not reduced). Moreover, whereas the degree of antibody reduction after IVIG has been shown to be time-dependent with

### TABLE 4. Pretransplant Antibody Work-Up (0–4 weeks): Anti-HLA Class I and II Specificity, and Donor Specific Anti-HLA Antibodies

<table>
<thead>
<tr>
<th>PT</th>
<th>Class I (%)</th>
<th>Major Specificities</th>
<th>Class II (%)</th>
<th>Major Specificities</th>
<th>Donor Specific Anti-HLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1†</td>
<td>0</td>
<td>POS‡</td>
<td>Anti-DR 5(11,12); DR 3 (17,18); DR13; DQ7</td>
<td>Anti-DR11, DR17</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>13</td>
<td>Anti-DR7</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>3†</td>
<td>44</td>
<td>Anti-A30, A31,A30; B44, B45</td>
<td>39</td>
<td>Anti-DR7, DR4, DR9</td>
<td>N/A</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>Anti-B15 (62,63,71,70); B21 (49,50); A33; A25</td>
<td>67</td>
<td>Anti-DR4, DR7,DR 9, DR53</td>
<td>Anti-DR4,DR7</td>
</tr>
<tr>
<td>5†</td>
<td>38</td>
<td>Anti-B7, B8</td>
<td>80</td>
<td>Anti-DR1, DR7, DR13, DR15, DR16, DR9, DR11</td>
<td>Anti-B8; DR1, DR13</td>
</tr>
<tr>
<td>6†</td>
<td>88</td>
<td>Anti-A1, A25, A26, A11, A34</td>
<td>90</td>
<td>Anti-DR11, DR7, DR9, DR13</td>
<td>Anti-DR11,13</td>
</tr>
<tr>
<td>7†</td>
<td>82</td>
<td>strong Anti-A2</td>
<td>64</td>
<td>Anti-DR1;DR13;DR15</td>
<td>Anti-A2; DR13,DR15</td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>Anti-A2, A68</td>
<td>0</td>
<td>Anti-A2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>47</td>
<td>Anti-DR5 (11,12); DQ7</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>70</td>
<td>Anti-DR4; DR3; DR5 (11,12);DR17, DR18</td>
<td>Anti-DR4</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>88</td>
<td>multispecific; dominant anti-A2</td>
<td>33</td>
<td>Anti-DR1,DR10</td>
<td>Anti-A2</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>30</td>
<td>Anti-DR3(17,18), DR13</td>
<td>Anti-DR13</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>58</td>
<td>Anti-B15 (B62, 63); B17 (57,58); A25, A32</td>
<td>90</td>
<td>Anti-DR1; DR3(17,18); DR5(11,12); DR13</td>
<td>Anti-DR13, B15</td>
</tr>
</tbody>
</table>

Results are those closest to transplant. All numeric PRA values were obtained using solid-phase Flow beads.

ND, none detected; N/A, donor typing not available.

*Positive B-cell XM; unable to determine specificity of donor-antigen specific antibody.
†Results following pre-treatment with IVIG and/or MMF.
‡Class II PRA values not available with then current technology.

### TABLE 5. "Pre-transplant" Anti-HLA Antibody Screen Before and After Pretreatment With IVIG (1 g/kg) ± MMF (20 mg/kg/d)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pretransplant therapy</th>
<th>Pretreatment Highest Value (%)</th>
<th>Post-treatment (%) Result closest to TX Class I/II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Weekly IVG x 3 weeks, MMF x 1 week (within 3 weeks before Htx)</td>
<td>0/43</td>
<td>0/POS*</td>
</tr>
<tr>
<td>3</td>
<td>Weekly IVG (3 doses-stopped 2 weeks pre-Tx)</td>
<td>46/43</td>
<td>44/39</td>
</tr>
<tr>
<td>5</td>
<td>IVIG –1 dose given 5 weeks pre-Tx; MMF × 5 weeks before HTx</td>
<td>56/85</td>
<td>38/80</td>
</tr>
<tr>
<td>6</td>
<td>IVIG –1 dose given 10 weeks pre-Tx; MMF × 10 weeks prior to HTx</td>
<td>90/73</td>
<td>88/90</td>
</tr>
<tr>
<td>7</td>
<td>IVIG—1 dose given 4 weeks pre-Tx; MMF × 4 weeks before HTx</td>
<td>100/100</td>
<td>82/64</td>
</tr>
</tbody>
</table>

Class II PRA not available but the specimen was anti-Class II positive by ELISA
maximal reduction at 1 week after treatment. Higher doses have been shown to have a more permanent effect on antibody reduction, and continued use after transplant might be effective because of its potent anti-inflammatory effect where antibody-mediated mechanisms are present. Nonetheless, even with pretreatment, continuing interventions after transplantation are clearly required to control rebound B-cell and plasma cell activity to prevent AMR as has been reported by others. Based on these results, listing for transplantation should not be delayed in the expectation that pretreatment will decrease PRAs, nor that it will reduce incidence of early humoral rejection; rather, patients can be effectively managed with B-cell therapeutic treatment strategies.

Recent reports have shown that rituximab treatment before transplant may help to decrease circulating preformed cytotoxic antibodies. Given our positive experience with rituximab initially on detection of AMR and, most recently in a preemptive manner perioperatively in sensitized patients, the move to pretreatment is the next logical step programmatically.

Rejection
Di Filippo and colleagues reported on the impact of ELISA-detected anti-HLA antibodies on cardiac allograft outcome in 45 pediatric patients (22/45 had pre- or post-HTx anti-HLA antibodies) and found that presensitization was more frequent in rejectors (ACR) in the first year post-HTx. We also found ACR and AMR to be a common problem early post-transplant in our patient population despite aggressive therapy. However, the majority of cases of early AMR and ACR were controlled with intensive immune modulation, and by 6 months post-HTx all cases had resolved. Moreover, these patients were able to wean from extensive B-cell directed therapies and were maintained by standard immunosuppressive regimens (eg, tacrolimus, MMF) similar to our nonsensitized patients. Interestingly, as stated, AMR has not been detected in follow-up despite the documented return of donor-specific anti-HLA antibodies. Whether this reflects some form of graft accommodation is unclear at the present time.

Graft Vasculopathy
In the Di Filippo study, 4/8 cases in their group with CAV had preformed anti-HLA antibodies compared with 8/37 without CAV (P=0.09). Other reports in the literature, both adult and pediatric, mirror this finding of a higher incidence of CAV associated with sensitization, a positive donor-specific crossmatch, or AMR. Intermediate data showing the lack of CAV development in the majority of our cohort is encouraging but longer follow-up is imperative before drawing any overall conclusions.

Infections and PTLD
Given the much higher level of early immunosuppression in this cohort, the comparable infection rates and the lack of transplant-related lymphomas is very encouraging. Nonetheless, the contribution of B-cell treatment on the subsequent longer-term development of graft vasculopathy and PTLD remains to be seen in this cohort, but is being closely monitored.

Limitations
Although this study is limited by its retrospective nature and a management strategy that evolved over time, it is one of the largest single-center experiences of pediatric heart transplantation in sensitized patients.

Future Directions
The future is encouraging for this group, with rapidly evolving detection and management technologies on the horizon. Recently, class 1 antibody analysis has expanded to include HLA-C, and class II to include HLA-DP. A level of prospective crossmatching has also been facilitated.
with the advent of the virtual cross-match at our institution and is now readily used. Recent research has also demonstrated that the assessment of post-transplant HLA-directed antibodies, particularly Class II antibodies related to allograft failure can affect outcome and modulate treatment strategies. During this study, we identified 2 additional patients that had a positive B-cell crossmatch, but no evidence of any anti-Class II antibodies (data not presented). This positive crossmatch was likely attributable to a non-HLA donor specific antigen. Novel technologies may allow us to define additional B-specific antigens that may influence patient outcomes and the course of treatment of such patients.

Overall Summary
Reasonable intermediate-term results are achievable in highly sensitized pediatric heart transplant recipients with positive donor-specific crossmatches using an algorithm of B cell-directed strategies pre- and post-transplant. However, it remains unclear whether the benefits of transplantation outweigh the risks of AMR, the morbidities associated with managing these patients in the long term, and overall survival. Prospective trials are required to assess evolving treatments directed at reducing preformed antibodies and immune-modulating strategies that may facilitate the management and optimize the outcome of this growing high risk population.

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Disclosures
None.

References
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