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Antibody Depletion for the Treatment of Crossmatch Positive Pediatric Heart Transplant Recipients

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Abstract

Sensitization to human leukocyte antigens (HLA) is a risk factor for adverse outcomes after heart transplantation. Requiring a negative prospective crossmatch results in longer waiting times and increased waitlist mortality. We report outcomes in a cohort of sensitized children who underwent transplant despite a positive complement dependent cytotoxicity (CDC) crossmatch (CM+) using a protocol of antibody depletion at time of transplant, followed by serial intravenous immunoglobulin administration. All patients less than 21 years old who underwent heart transplantation at Boston Children's Hospital from 1/1998-1/2011 were included. We compared freedom from allograft loss, allograft rejection, and serious infection between CM+ and CM- recipients. Of 134 patients in the cohort, 33 (25%) were sensitized prior to transplantation and 12 (9%) received a CM+ heart transplant. Serious infection in the first post-transplant year was more prevalent in the CM+ patients compared to CM- patients (50% vs. 16%; $P=0.005$), as was hemodynamically significant antibody mediated rejection (50% vs. 2%; $P<0.001$). There was no difference in freedom from allograft loss or any rejection. At our center, children transplanted despite a positive crossmatch had acceptable allograft survival and risk of any rejection, but a higher risk of hemodynamically significant antibody-mediated rejection and serious infection.

Keywords

pediatric heart transplantation; anti-HLA antibody; plasmapheresis; outcome; infectious risk; antibody mediated rejection

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Author Contributions

KPD, SFC, CSA, TPS and EDB were responsible for study design, data analysis/interpretation, and critical revision of the article. KPD and SFC were responsible for drafting the article. HM, EM, GSM, HJB, JEM, and FFT were responsible for data interpretation and critical revision of the article. All authors approved the final submission. We would like to acknowledge Peter MacAskill for help obtaining the DSA MFI data.

INTRODUCTION

Sensitization to human leukocyte antigens (HLA) has historically presented a difficult challenge to successful and timely heart transplantation. Children with complex congenital heart disease who have failed surgical palliation represent an important, but challenging, group of transplant recipients.(1–6) These children have often been exposed to homograft material, multiple blood transfusions, and/or ventricular assist devices, all of which contribute to the development of anti-HLA antibodies.(7–11)

Traditionally, heart transplant candidates with an elevated complement-dependent cytotoxic (CDC) panel-reactive antibody (PRA) have been listed with the requirement for a negative prospective donor-specific T-cell crossmatch, as transplant recipients with a CDC positive crossmatch have worse allograft survival.(2, 3, 4, 12–15) This strategy has been shown to increase time on the wait list and wait list mortality for pediatric and adult heart transplant recipients.(16, 17) Clinical protocols have been developed for peri-operative antibody removal via plasmapheresis or plasma exchange accompanied by induction therapy, post-transplant IVIG, and ‘triple’ drug immunotherapy.(16, 18–23) By decreasing the titers of donor-specific anti-HLA antibodies, these protocols were designed to decrease the risk of rejection and improve allograft survival while allowing for an expansion of the donor pool.(23, 24)

There is limited published data on allograft survival after pediatric heart transplantation against a positive CDC T-cell crossmatch (CM+). Even less is known about how antibody depletion and replacement may modify those outcomes. In this single institution retrospective study, we compare allograft survival, time to rejection, and time to infection in CDC crossmatch positive pediatric patients who underwent a standard peri-transplant antibody depletion protocol to a control cohort of CDC crossmatch negative heart transplant recipients who received standard immunosuppressive therapy.

METHODS

Subjects

We retrospectively reviewed the charts of all patients who were less than 21 years of age at the time of a heart transplant procedure at Boston Children’s Hospital between 7/1/1998 and 1/31/2011 (n=141). A total of 12 patients had undergone heart transplantation despite a positive CDC-CM against donor T- or B-cells. Patients with a negative donor specific CDC-CM formed the control cohort (n=122). Three patients who did not have a donor specific CDC-CM performed were excluded from analysis. Four patients underwent two heart transplant procedures during the study period; only one procedure was included per patient in the following order of prioritization: (1) the CDC-CM positive procedure; (2) the earliest procedure in the study period. Baseline pre-transplant variables, donor data, peri-operative therapies, and post-operative events (allograft survival, allograft rejection, serious infection) were collected and analyzed. This study was approved by the Committee on Clinical Investigation at Boston Children’s Hospital. All study subjects, or their parents/legal guardians, provided written consent prior to transplantation for collection of clinical data.

Human Leukocyte Antigen (HLA) Sensitization and Donor Specific Antibody (DSA) Detection

At the beginning of the study period, T-lymphocyte Panel Reactive Antibody (PRA) levels were determined by utilizing the AHG (anti-human globulin) complement dependent lymphocytotoxicity method. In this method, a prospective candidate’s serum sample is incubated with T-lymphocytes from multiple representative donors covering the most prevalent HLA class I specificities. The PRA level represents the percentage of donor

samples against which the potential recipient sample induced a positive lymphocytotoxic response. In 2006, the primary method for detecting anti-HLA antibody changed in our tissue typing lab from the CDC-PRA assay to a single antigen bead-based method (One Lambda, Canoga Park, CA). A mean fluorescence intensity (MFI) cutoff of 1000 was utilized and single antigen specificities were transformed to a calculated PRA (cPRA) based on the HLA antibodies detected in the assay and the known prevalence in the United States population using an algorithm developed by the Organ Procurement & Transplantation Network (OPTN).(25) Patients whose pre-transplant CDC-PRA or cPRA was greater than 10% were considered sensitized to HLA antigens. Peri-and post-transplantation, antibodies were reviewed and compared to donor HLA serotyping to determine if DSA were present. Serial peak MFI data is provided for all DSAs in the CM+ recipients. One Lambda C1qScreen was used to identify the presence of complement fixing antibody in two patients at most recent follow up.

Antibody Depletion and Immune Modulation

Four of the 33 sensitized patients were listed with a requirement for a negative prospective crossmatch and received a crossmatch negative donor allograft by design. The remaining sensitized patients received a transplant before the donor specific crossmatch results were available. Pre-operative antibody depletion was performed in all sensitized patients via a 1.5–2.0 volume exchange by either plasmapheresis or plasma exchange upon initiation of cardiopulmonary bypass. A CDC-CM was performed contemporaneously with the transplant surgical procedure for all sensitized patients. If either the CDC T-cell or B-cell crossmatch was positive, plasmapheresis was continued daily or on alternating days for 4–6 cycles following transplant. Each plasmapheresis cycle consisted of a 1.5–2.0 volume plasma exchange utilizing FFP (10–15mL/kg) for replenishment of clotting factors and 5% albumin for volume replacement. Intravenous immunoglobulin (IVIG) was administered (2 g/kg) 6–10 days post-transplant and every 3–4 weeks thereafter for 6–12 months. For sensitized patients with a negative CDC donor specific crossmatch, plasmapheresis was not continued in the post-transplant period nor was IVIG routinely administered.

Standard Lymphocyte Targeted Immunomodulatory Therapy and Rejection Surveillance

From July 1998 through April 2005 all patients received standard post-transplant triple agent immunotherapy consisting of (1) cyclosporine, (2) azathioprine or mycophenolate mofetil (MMF), and (3) prednisone without induction therapy. Starting in April 2005 all heart transplant recipients received induction therapy with anti-thymocyte globulin (ATG; 1.5 g/kg/dose daily for 5 days). Patients were then maintained on tacrolimus and MMF starting on post-transplant day 2–4. Only patients with a positive crossmatch were maintained on prednisone.

Protocol endomyocardial biopsies were performed at the following time points post-transplantation: Weeks 2, 4, 6, 9, 12, 25, 38, and 52. In all patients with a positive CDC T-cell crossmatch, endomyocardial biopsies were routinely evaluated using C4d immunofluorescence alone to assist in the diagnosis of antibody-mediated rejection. This test became routinely available at our center in February 2003.

Outcome Variable

The primary outcome variable was cardiac allograft loss (death or re-transplantation). Patients who did not experience allograft loss were censored at last follow up. As of November 30, 2012, 2 patients were lost to follow up, 19 patients had transitioned to another transplant center, and 72 were censored at their last clinic visit. We evaluated the following secondary outcomes: (1) time to first rejection episode and (2) time to first infection episode. Rejection episodes were defined as either (1) an endomyocardial biopsy showing ISHLT

grade > 2R cellular rejection (old grade >3A) or (2) hemodynamically significant antibody mediated rejection (HD-AMR).(13, 26) The following criteria were used for hemodynamically significant antibody mediated rejection: (1) graft dysfunction (systolic dysfunction or acute elevation in pulmonary capillary wedge pressure) without cellular rejection, (2) presence of circulating donor-specific antibody, (3) positive C4d immunofluorescence, and (4) endothelial swelling with interstitial edema on light microscopy.(26) Prior to release of the 2005 ISHLT guidelines, HD-AMR was defined as systolic graft dysfunction in the presence of a biopsy negative for acute cellular rejection but positive for endothelial swelling.

An episode of infection was defined as an infection which required IV antibiotic treatment or hospital admission. All recorded episodes of infection included a complete treatment course of antibiotics and a documented infection either by blood, urine, wound, cerebrospinal fluid or tissue culture/biopsy.

Statistical Analysis

Patient characteristics are reported as median with ranges. Comparisons between groups were performed using Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables. Kaplan-Meier survival analysis was used to compare time to allograft loss, time to first rejection and time to first infection episode. Groups were compared using the log-rank test. All tests were two sided. The data were analyzed using STATA version 10.0 statistical software (StataCorp LP, College Station, TX).

RESULTS

Pre-Transplant Patient Characteristics

Thirty-three of the 134 (25%) patients were HLA sensitized, of whom 12 had a positive donor specific CDC-CM at the time of transplant. Both the T- and B-cell CDC-CM were positive in 8 patients, the B-cell was positive and T-cell was negative in 2, and the T-cell was positive and B-cell was not performed in 2. Of the twelve CM+ patients, the median age was 6.1 years (range: 0.4–15.9) and 6 were female (50%). When compared to the group of CM– patients, CM+ patients had significant differences in pre-transplant diagnosis, prior sternotomy, and HLA sensitization status (Table 1; all $P<0.001$). The majority of CM+ patients had congenital heart disease while the majority of the CM– patients had a diagnosis of cardiomyopathy. All of the CM+ patients had a history of prior sternotomy compared to 43% of the CM– patients ($P=0.001$). CM+ patients had significantly more HLA sensitization than CM– patients. Of the 10 CM+ patients who had undergone single antigen bead screening, the cPRA was $63\% \pm 38$ for HLA Class I antibody and $63\% \pm 35$ for HLA Class II antibody.

Donor Specific Antibody

HLA sensitization, donor specific crossmatch results, and DSA results for the 12 CM+ recipients are summarized in Table 2. Eight CM+ patients had single antigen bead DSA data available starting at the time of transplant, five of whom had at least one DSA with a measured MFI greater than 5000. An additional three CM+ patients had DSA data available at follow up. Ten of the CM+ patients had known DSA present at the time of transplant. The other two had quick screen results of 45% and 52% with unknown single antigen specificities and a positive donor specific T-cell crossmatch. Plasmapheresis did not result in significant decreases in the number or peak MFI of DSA for most patients. However, after completion of IVIG, there were decreases in the number of DSA, peak MFI, and peak MFI for the strongest antibody at the time of transplant for most CM+ patients (Table 2). All patients who survived beyond 5 years, or are still alive at last follow up, showed continued decreases

in the number of DSA and peak MFI for each DSA at last follow up. One patient (#10) developed 3 *de novo* Class I DSA in the first six months after transplant and another (#7) developed 2 *de novo* Class II DSA 2 years after transplant. Both of these patients died within months of developing *de novo* DSA.

Allograft Loss and Rejection

There were no significant differences in allograft survival between CM+ patients and CM- patients (Figure 1; $P=0.20$). In the first post-transplant year, CM+ patients were not more likely to experience rejection compared to non-sensitized patients treated with a standard protocol (Table 3; 50% vs. 39%; $P=0.47$). There were also no significant differences in time to first rejection (Figure 2; $P=0.30$) nor in the number of rejection episodes in the first year (1 [1,1] vs. 1 [0,1]; $P=0.17$). After ATG induction was introduced into our practice, there was no change in allograft survival; However, there was a decrease in the median number of rejection episodes in the first post-transplant year from 1 to 0 ($P=0.001$).

However, HD-AMR during the first post-transplant year was more frequent in the CM+ patient population, occurring in 50% of these patients compared to 2% of CM- patients (Table 3). All patients with HD-AMR had DSA detectible by either single antigen bead testing or a positive retrospective donor specific crossmatch before such testing became available. Episodes of HD-AMR rejection were treated with pulse dose steroids and plasmapheresis in all cases. In addition, three of the six episodes were treated with IVIG and one was treated with IVIG and rituximab. Two CM+ patients developed *de novo* DSA (one in the context of medication non-compliance) and experienced sudden death shortly thereafter (Table 2). A third CM+ patient experienced early HD-AMR, was treated with plasmapheresis, IVIG, and rituximab and discharged home. She died suddenly and was found to have severe acute cellular rejection (ACR) with diffuse CAV at autopsy. The final early death in the CM+ cohort was due to primary graft failure leading to multi-system organ failure. There was no evidence of AMR and no response to plasmapheresis. Autopsy revealed ischemic necrosis of the heart but no evidence of ACR or AMR.

Infection

There were significantly more treated infections during the first post-transplant year in the CM+ patients compared to CM- patients (Table 3; 50% vs. 16%; $P=0.005$). CM+ patients had significantly shorter time to first infection than CM- patients (Figure 3; $P<0.001$). The six CM+ patients were treated for either one ($n=4$) or two ($n=2$) episodes of infection. Infections included one case of HSV treated with acyclovir, two cases of *Clostridium difficile*, one episode of CMV myocarditis treated with ganciclovir, one episode of CMV reactivation treated with CMV specific immunoglobulin and IV ganciclovir, one episode of MSSA sternal wound infection, one episode of *Candida Albicans* mediastinitis treated with liposomal amphotericin B, and one episode of *Serratia marcescens* mediastinitis and sepsis. None of the patients died from their infections.

There were twenty CM- patients treated for a total of one ($n=9$), two ($n=3$), three ($n=5$), and four ($n=3$) infectious episodes in the first post-transplant year. Documented infections included the following: *Pneumocystis jiroveci*, *Cryptococcus*, *Mycobacterium avium*, Aseptic Meningitis, *C. difficile*, *Enterobacter*, *Serratia marcescens*, *H. influenza*, *S. epidermidis*, CMV, VRE, *C. albicans*, *C. krusei*, *S. aureus* sepsis, *Aspergillus*, *Histoplasma capsulatum*, and *Pseudomonas aeruginosa*.

Factors associated with serious infection during the first post-transplant year

Twenty-six patients were treated for a serious infection in the first post-transplant year. The strongest factor associated with serious infection within the first year was the patient having

a positive donor specific CDC-CM (Table 4). Per our center's protocol, all CM+ patients received intensive antibody depletion therapy. As expected, patients who underwent antibody depletion with plasmapheresis followed by IVIG replacement accounted for a larger proportion of the serious infections but this did not reach statistical significance ($P=0.11$). Patients who received ATG induction therapy were not more likely to require therapy were not more likely to require treatment for a serious infection ($P=0.63$).

DISCUSSION

Children undergoing orthotopic heart transplantation despite a positive CDC T-cell or B-cell crossmatch treated with a standardized peri-operative antibody depletion protocol and IVIG replacement do not show differences in mid-term allograft survival compared to crossmatch negative patients. While CM+ patients were more susceptible to serious infection, this is most likely due to the use of an intensive antibody depletion protocol rather than pre-transplant allosensitization *per se*. There was no difference in time to first rejection between the CM+ cohort and the CM- controls; however, episodes of hemodynamically significant antibody mediated rejection were significantly more likely to occur in the CM+ cohort.

Historically, highly-sensitized patients have been listed for heart transplant with the requirement for a negative donor specific crossmatch prior to transplantation; a requirement predicated by data suggesting that the presence of positive CDC crossmatch was associated with worse outcomes.(2, 4, 12, 15) Although prudent when considering post-transplant outcomes, this requirement has adverse consequences for patients prior to transplant. Feingold et al. (27) reported that when compared to non-sensitized patients, sensitized subjects (pre-HTx PRA>10%) were significantly more likely to die on the wait list (22% vs. 8%). Despite this data, the number of pediatric patients listed with a requirement for a negative crossmatch (either donor specific or virtual) has increased in the last 15 years.(17)

Allosensitization is becoming increasingly common as more pediatric heart transplant candidates have been supported with ventricular assist devices or have undergone palliative surgery for congenital heart disease.(9, 11, 28) In addition, sensitive laboratory methods for detecting alloantibody have led to an apparent increase in the number of sensitized patients. Whether this increase represents important clinical information which can be used to improve transplant outcomes or a test with a high false positive rate remains to be seen.

Our results substantiate those in other small series (29, 30) which suggest that short-term survival for those patients with a CDC-positive donor specific crossmatch is similar to those with a negative crossmatch. However, the current study differs from that of Holt *et.al* in several important ways. First, our treatment protocol did not use cyclophosphamide and instead followed plasmapheresis with IVIG infusions every 3–4 weeks for 6–12 months. We have demonstrated a decrease in DSA over time during IVIG therapy. We did not see significant changes in DSA with plasmapheresis alone. This strategy achieved acceptable outcomes, even in patients with DSA MFI values over 5000 at the time of transplantation. In addition, we demonstrated that these patients are at higher risk for infection. These data illustrate the delicate balance that must be struck between preventing graft rejection and limiting susceptibility to infection.

A retrospective multicenter study from the Pediatric Heart Transplant Study group and preliminary data from the OPTN database have shown that allograft survival is worse in patients with a positive donor specific crossmatch.(31, 32) The similar allograft outcomes in our series could be the result of a study that was underpowered to detect small, but important differences in mid-term allograft survival. Alternatively, it may suggest that aggressive treatment with an antibody depletion protocol followed by IVIG replacement can improve

allograft survival for recipients of CDC crossmatch positive patients. In a resource limited population, such as the highly sensitized pediatric cardiac recipient, we believe that transplantation against a positive crossmatch is an acceptable strategy. Patients and their families should be made aware of the increased risks of AMR and infection and the risks/benefits of alternative strategies should be discussed. However, transplantation against a positive crossmatch using antibody depleting protocols appears to be a prudent and safe strategy for optimizing the chances of receiving a donor heart in critically ill highly sensitized pediatric transplant candidates. Larger prospective multicenter studies are necessary to properly define the effects of a positive crossmatch on pediatric post-transplant outcomes and to specifically identify which crossmatch method performs best in risk stratifying recipients.

Our data demonstrate that CM+ patients are at increased risk of hemodynamically significant AMR early post-transplantation. Frequent AMR screening in early post-transplant period for CM+ patients should allow earlier AMR identification and provide a therapeutic window to modulate immunotherapy. Screening should include C4d staining of endomyocardial biopsy samples and periodic assessment for donor specific antibody via solid phase techniques.(33)

Risks of the Protocol

In our cohort, the number of treated infections was higher in CM+ pediatric heart transplant recipients compared to the CM- controls. This is most likely a direct effect of the antibody depletion protocol which indiscriminately eliminates antimicrobial antibodies as well as anti-HLA antibodies. Severe infection remains an important cause of death in pediatric heart transplant recipients.(34) The current study suggests that reconsideration of the most effective methods for preventing and managing infections in the early post-transplant period may be warranted in patients treated with an antibody depletion protocol.

Limitations

It is important to note that this study is limited by the retrospective nature of the data collection. We used a HD-AMR definition that combined clinical and pathologic characteristics which may limit the ability of clinicians to apply the AMR findings to their patients. Future prospective studies should apply the ISHLT working formulation for pathologic diagnosis of AMR in order to increase generalizability of the findings (33). The number of sensitized patients with a positive crossmatch was small, which increases the uncertainty of the point estimates. Our study is prone to type II error and an inability to detect a true difference of small to moderate magnitude due to small sample size. This limitation is balanced by the fact that a similar protocol for antibody depletion was used in all crossmatch positive patients, which may account for the lack of differences in allograft survival that were observed. Midway through the study period, our center shifted to ATG induction therapy in all patients along with elimination of maintenance corticosteroids for CM- patients. This did not result in a change in allograft survival, but it did decrease the incidence of cellular rejection. Since a slightly higher proportion of CM+ patients received ATG induction than the overall population, it is possible that ATG induction therapy contributed to successful transplantation despite a positive CM. We were unable to adjust for potential confounders, such as congenital heart disease, in our analysis due to the small sample size. Finally, we assumed that the presence of either a positive T- or B-cell donor specific crossmatch conferred a similar immunologic risk to the allograft.

Conclusions

In recent years, our strategy of requiring a negative prospective crossmatch in sensitized patients has been altered in the face of new antibody depletion protocols. This strategy was

developed in order to reduce wait list times, and hence wait list mortality for this historically disadvantaged group. For pediatric patients transplanted in the presence of a positive donor specific CDC crossmatch, antibody depletion immediately prior to heart transplant followed by post-operative plasmapheresis and IVIG results in acceptable mid-term survival and cellular rejection profiles similar to those seen in crossmatch negative patients. This study further illustrates that crossmatch positive patients treated with antibody depletion are at increased risk of serious infection. A high index of clinical suspicion for infection should be maintained, and intensive surveillance for treatable infections should be performed, for these patients. Larger multi-center studies are needed to better understand the impact of sensitization on pediatric heart transplant outcomes and the long term effects of such antibody depletion therapy.

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Figure 1. Kaplan-Meier freedom from allograft loss after pediatric heart transplantation stratified by CDC crossmatch result. There was no significant difference in allograft loss ($P=0.2$).

Figure 2.

Kaplan-Meier freedom from rejection (ISHLT Grade 3A/2R cellular rejection or antibody mediated rejection) after pediatric heart transplantation stratified by CDC crossmatch result. There was no significant difference in the time to first rejection episode ($P=0.3$).

Figure 3. Kaplan-Meier freedom from serious infection after pediatric heart transplantation stratified by CDC crossmatch result. Patients with a positive CDC crossmatch were significantly more likely to be treated for infection ($P<0.001$).

Table 1

Patient, transplant, and treatment characteristics

Variable	Crossmatch Positive (n=12)	Crossmatch Negative (n=122)	P-value
Patient characteristics			
Female sex	6 (50%)	52 (43%)	0.62
Caucasian race	9 (82%)	100 (82%)	0.99
Median age at transplant (y) (range)	6.1 (0.4–15.9)	8.8 (0.02–19.3)	0.58
Pre-transplant diagnosis			<0.001
Cardiomyopathy	1 (8%)	69 (56%)	
Congenital heart disease	8 (67%)	50 (41%)	
Transplant allograft dysfunction	3 (25%)	2 (2%)	
Myocarditis	0 (0%)	1 (1%)	
Prior cardiac surgery	12 (100%)	55 (45%)	<0.001
HLA sensitization pre-transplant	12 (100%)	21 (17%)	<0.001
cPRA to HLA class I (n=52)	80 [21,90]	0 [0, 8]	<0.001
cPRA to HLA class II (n=52)	71 [39, 95]	0 [0, 0]	<0.001
Transplant characteristics			
Date of transplant			0.20
1998 – 2002	2 (17%)	48 (39%)	
2003 – 2006	3 (25%)	32 (26%)	
2007 – 2011	7 (58%)	42 (35%)	
Treatment characteristics			
Peri-transplant therapy			
ATG induction	7 (58%)	45 (37%)	0.15
Plasmapheresis	12 (100%)	6 (5%)	<0.001
IVIg	11 (92%)	11 (9%)	<0.001
Baseline immunosuppression			
Tacrolimus	9 (75%)	61 (50%)	0.1
Cyclosporine	2 (17%)	59 (48%)	0.04
Azathioprine	1 (8%)	58 (48%)	0.01
Mycophenolate mofetil	11 (92%)	62 (51%)	0.01
Prednisolone/Prednisone	10 (91%)	78 (65%)	0.08

HLA, human leukocyte antigen; cPRA, calculated panel reactive antibody; ATG, anti-thymocyte globulin; IVIG, intravenous immunoglobulin; HLA Sensitization defined as having a CDC-PRA or cPRA >10%

HLA sensitization, crossmatch results, donor specific antibody characteristics, and outcome for all crossmatch positive patients

Table 2

Patient	cPRA		Virtual “Crossmatch”		Crossmatch		Post-Plasmapheresis		6 Months Post-Tx		Most Recent Follow Up		Patient Status, Time Post-Tx; Notes
	Class I	Class II	Class I DSA [MFI]	Class II DSA [MFI]	T-Cell	B-Cell	Class I DSA [MFI]	Class II DSA [MFI]	Class I DSA [MFI]	Class II DSA [MFI]	Class I DSA [MFI]	Class II DSA [MFI]	
1	70 (Qk)		1 DSA (Qk)	Unknown	Pos	Unk	100% (Qk)		0% (Qk)		Negative		Alive, 15 y; Early AMR
2	45 (Qk)		45% (Qk)	Unknown	Pos	Pos			66% (F)	99% (F)			Alive, 10 y;
3	80 (F)	10 (F)	1 DSA (Qk)	Unknown	Pos	Unk	1 [Unk]	0	0	0	Negative		Died, 7 y; Early AMR Cause unknown
4	86 (F)	48 (F)	52% (Qk)	Unknown	Pos	Pos	27% (Qk)		2% (Qk)		Negative		Alive, 9 y;
5	68	78	1 [6700]	1 [12000]	Pos	Pos	1 [Unk]	1 [2000]	NA	NA	1 [Unk]	1 [2000]	Died, 2 w; Ischemic necrosis of heart, MSOF, No ACR.
6	79	71	2 [2807, 1634]	0	Pos	Pos	2 [5300, 1074]	1 [2045]	1 [2207, Neg]	0	1 [1351]	0	Alive, 6 y; Early AMR
7	4	71	0	1 [2927]	Neg	Pos	0	1 [3815]	0	0	0	3 [6512, 1250, 1154]; 2 <i>de novo</i>	Died, 2.5 y; 2 <i>de novo</i> Class II DSA 2 y post-tx at time of AMR. Developed severe CAV and died suddenly.
8	88	94	0	2 [10400, 2651]	Pos	Pos	0	2 [2711, 13484]	0	1 [Neg, 6881]	0	1 [Neg, 12401]	Alive, 6 y; C1q negative at last follow up.
9	26	0	1 [8390]	0	Pos	Pos	1 [1445]	5 [1375, 1366, 1317, 1040, 1018]	0	0	0	0	Alive, 1.5 y;
10	88	94	0	2 [4509, 4206]	Pos	Pos	0	2 [14825, 1252]	3 [8837, 5489, 3009]; All <i>de novo</i>	2 [3540, 8172]	3 [8837, 5489, 3009]; All <i>de novo</i>	2 [3540, 8172]	Died, 9 m; 3 <i>de novo</i> Class I DSA 6 m post-tx with AMR. Sudden cardiac death.
11	100	99	4 [9690, 8500, 3540, 1879]	5 [16029, 11916, 11262, 9452, 6068]	Pos	Pos	4 [6314, 6464, 18928, 18928]	6 [7723, 5009, 5306, 14848, 14193, 3982]	2 [Neg, Neg, 9483, 4169]	5 [1222, 1461, 2775, 4673, 2775, Neg]	1 [Neg, Neg, 3893, Neg]	1 [Neg, Neg, Neg, 2035, Neg]	Alive, 4 y; C1q negative at last follow up.
12	96	100	2 [8803, 2734]	5 [21263, 20805, 19988, 15854, 10713]	Neg	Pos	1 [2264, Neg]	5 [18003, 10146, 7730, 10982, 1905]	NA	NA	0	4 [17566, 3178, 3628, 8800, Neg]	Died, 2 m; Early AMR, Severe acute cellular rejection with diffuse CAV at autopsy.

HLA, human leukocyte antigen; cPRA, calculated panel reactive antibody; Tx, transplant; MFI, mean fluorescent intensity; Qk, Anti-kappa quick screen result; DSA, donor specific antibody; F, FlowPRA screen result; MSOF, multi-system organ failure; AMR, antibody mediated rejection; CAV, cardiac allograft vasculopathy;

Note: When available, MFI data is given for each DSA. The peak MFI to any given donor specific HLA serotype is reported. The order of the MFI data was kept consistent across each row so that values for a given DSA can be compared. Any *de novo* DSA were added to the end of the list.

Table 3

Outcomes in the first post-transplant year stratified by crossmatch results

Outcome	Crossmatch Positive (n=12)	Crossmatch Negative (n=122)	P-value
Rejection			
Any Rejection	6 (50%)	48 (39%)	0.47
HD-AMR	6 (50%)	2 (2%)	<0.001
Serious Infection	6 (50%)	20 (16%)	0.005
Allograft Loss	3 (25%)	12 (10%)	0.11

Any Rejection defined as ISHLT cellular rejection Grade 3A/2R or antibody mediated rejection; HD-AMR, hemodynamically significant antibody mediated rejection

Table 4

Transplant and treatment characteristics stratified by the presence of serious infection in the first post-transplant year

Transplant/Treatment Characteristics	Serious Infection (n=26)	No Infection (n=108)	P-value
CDC-crossmatch positive	6 (23%)	6 (6%)	0.005
ATG induction therapy	9 (35%)	43 (40%)	0.63
Plasmapheresis with IVIG replacement in first year	6 (23%)	12 (11%)	0.11
Prednisone/prednisolone, 30 days post-transplantation	19 (79%)	64 (62%)	0.11

CDC, complement mediated cytotoxicity; ATG, anti-thymocyte globulin; IVIG, intravenous immunoglobulin