DR. SETH A HOLLANDER (Orcid ID : 0000-0002-0818-3150)

DR. DAVID M PENG (Orcid ID : 0000-0001-7763-7518)

Article type : Original

PATHOLOGICAL ANTIBODY-MEDIATED REJECTION IN PEDIATRIC HEART TRANSPLANT RECIPIENTS: IMMUNOLOGIC RISK FACTORS, HEMODYNAMIC SIGNIFICANCE, AND OUTCOMES.

Seth A. Hollander MD[1]

David M. Peng MD[2]

Marcos Mills MD[1]

Gerald J. Berry MD[3]

Marny Fedrigo MD[4]

Doff B. McElhinney MD[5]

Christopher S. Almond MD[1]

David N. Rosenthal MD[1]

[1] Department of Pediatrics (Cardiology), Stanford University School of Medicine

[2] Department of Pediatrics (Cardiology), University of Michigan School of Medicine

[3]Department of Pathology, Stanford University School of Medicine

[4] Department of Cardiac Thoracic and Vascular Sciences, University of Padua Medical School, Padua, Italy

[5] Department of Cardiothoracic Surgery, LPCH Heart Center Clinical and Translational Research Program

Drs. Hollander and Peng contributed equally to this study and share first authorship.

Please send correspondence to:

Seth Hollander, MD

Clinical Assistant Professor, Pediatrics (Cardiology)

Stanford University

750 Welch Road, Suite #305

Palo Alto, CA 94304

PHONE: 650-736-8600

FAX: 650-497-8422

seth.hollander@stanford.edu

Authorship Statements

Dr. Hollander designed the study, created the database, performed data collection and analysis, and wrote the manuscript.

Dr. Peng helped design the study, performed data collection, and edited the manuscript.

Dr. Mills performed data collection and edited the manuscript.

Dr. Berry interpreted all biopsy slides and edited the manuscript.

Dr. Fedrigo interpreted most biopsy slides and edited the manuscript.

Dr. McElhinney participated in study design, statistics, data interpretation, and edited the manuscript.

Dr. Almond participated in study design and edited the manuscript.

Dr. Rosenthal participated in study design, data interpretation, and edited the manuscript.

Abbreviations

ACR	Acute Cellular Rejection
AMR	Antibody-Mediated Rejection
CHD	Congenital Heart Disease
C1q+	C1q Positive
DSA	Donor-Specific Antibody
EMB	Endomyocardial Biopsy
CAV	Cardiac Allograft Vasculopathy
cPRA	Calculated Panel Reactive Antibody
HLA	Human Leukocyte Antigen
HT	Heart Transplant
min-max	Minimum-Maximum
Q1-Q3	Quartile 1- Quartile 3
IgG	Immunoglobulin G
IgG+	Immunoglobulin G Positive
ISHLT	International Society for Heart & Lung Transplantation
IVIG	Intravenous Immunoglobulin
LPCH	Lucile Packard Children's Hospital, Stanford
MFI	Mean Fluorescence Intensity
pAMR	Pathological Antibody-Mediated Rejection
pAMR1i	Pathological Antibody-Mediated Rejection Grade 1 (Immunohistochemistry)
pAMR2	Pathological Antibody-Mediated Rejection Grade 2
PCWP	Pulmonary Capillary Wedge Pressure
POD	Post-Operative Day
RAP	Right Atrial Pressure
VAD	Ventricular Assist Device
VXM	Virtual Crossmatch

Hollander SA, Peng DM, Mills M, Berry GJ, Fedrigo M, McElhinney DB, Almond CS, Rosenthal DN
PATHOLOGICAL ANTIBODY-MEDIATED REJECTION IN PEDIATRIC HEART TRANSPLANT RECIPIENTS: IMMUNOLOGIC RISK FACTORS, HEMODYNAMIC SIGNIFICANCE, AND OUTCOMES.
Pedtr

Abstract

Biopsy-diagnosed (pathological) antibody-mediated rejection (pAMR) has been observed in over half of pediatric heart transplant (HT) recipients within 6 years of transplantation. We report the incidence and outcomes of pAMR at our center. All endomyocardial biopsies for all HT recipients transplanted between 2010-2015 were reviewed and classified using contemporary ISHLT guidelines. Graft dysfunction was defined as a qualitative decrement in systolic function by echocardiogram or an increase of ≥3 mmHg in atrial filling pressure by direct measurement. Among 96 patients, pAMR2 occurred in 7 (7%) over a median follow-up period of 3.1 years, while no cases of pAMR3 occurred. A history of congenital heart disease, donor specific antibody (DSA) at transplant, and elevated filling pressures were associated with pAMR2. 5/6 (83%) of patients developed new C1q+ DSA at the time of pAMR diagnosis. There was a trend towards reduced survival, with 43% of patients dying within 2.3 years of pAMR diagnosis.

Key Words

Rejection
Heart
Hemodynamics
Antibody
Outcomes

Introduction

Although antibody-mediated rejection (AMR) in the cardiac allograft was first described in the late 1980s, broad acceptance of AMR as a distinct clinicopathological entity evolved more slowly.[1, 2] Early studies defined AMR using various combinations of clinical and pathological features, including the presence of graft dysfunction, emergence of donor specific antibody (DSA), and/or the clinical decision

to employ immunomodulatory therapies in the setting of suspected AMR in the absence of biopsy findings.[3-5] To address these inconsistencies, in 2011, the International Society for Heart & Lung Transplantation (ISHLT) published a consensus statement standardizing the nomenclature for the pathological findings of antibody-mediated rejection (pAMR), allowing for a more uniform description of its histological and immunohistochemical features, a descriptive and numeric grading scheme and, as with acute cellular rejection (ACR), providing a framework for the diagnosis of AMR independent of clinical, hemodynamic, or serological factors.[6, 7]

With a more consistent diagnostic criterion, there is now a growing body of literature in the adult population correlating pAMR severity with hemodynamic disturbance as well as patient and graft outcomes.[8-10] In the pediatric population, however, the prevalence, clinical profile, and prognostic significance of pAMR, are not as well described. To date, few studies have examined the incidence and outcomes of pAMR in children utilizing the 2011 criteria. In 2012, Everitt et al. reported that pAMR grade 2 or higher occurred in 18% of endomyocardial biopsies and 59% of pediatric heart transplant (HT) recipients, with severe (grade 3) pAMR in 1% of biopsies and 12% of patients.[11] Although the study did not uncover an association between pAMR2 and poor outcomes, pAMR3 was associated with a significantly lower freedom from cardiovascular related mortality or cardiac allograft vasculopathy (CAV) within 5 years of transplant.[11] More recent studies in updated cohorts report lower rates, with ≥pAMR2 occurring in of 21% of pediatric HT patients.[12]

The development of the current pAMR grading criteria also offers the opportunity to better examine the role of human leukocyte antibody (HLA) and pAMR development. In 2016, Ware, et. al demonstrated that the presence of DSA had excellent sensitivity and negative predictive value for biopsy-diagnosed AMR using the current criteria, although the study did not examine the role of complement fixation on pAMR development.[12] Since 2007, the pediatric heart transplant program at Stanford University has used the C1q assay to define the subset of HLA antibodies capable of fixing complement, as we believe complement fixing antibodies are more likely to precipitate myocardial injury.[13] Though C1q+ DSA have been associated with biopsy-diagnosed AMR using earlier definitions, the correlation between C1q+ DSA and pAMR in pediatric HT recipients using the current criteria has not been sufficiently examined.[14]

The purpose of this study was to ascertain the incidence of and risk factors for pAMR using the current grading criteria in a single-center cohort of pediatric HT recipients, and to correlate pAMR development with changes in immunologic risk factors, changes in ventricular function, and patient outcomes.

Materials & Methods

Study Population and Clinical Data Collection

We performed a retrospective chart review on all patients who underwent HT at Lucile Packard Children's Hospital, Stanford (LPCH) between January 1, 2010 and December 31, 2015 and who received at least one endomyocardial biopsy (EMB). The incidence and outcomes for early (≤1 year) or late (>1 year) pAMR were analyzed from the time of HT to December 31, 2016. Patients undergoing multi-organ (heart-liver) transplant were excluded.

Baseline demographics, including age, gender, race, and pre-transplant diagnosis, as well as all post-transplantation catheterization and EMB data were extracted from the electronic medical record. All EMB catheterizations were included, whether they occurred at LPCH or an outside hospital, however, biopsy results were only included if they were interpreted by a Stanford University cardiac pathologist. All biopsies underwent un-blinded review by an experienced cardiac pathologist at the time of the biopsy procedure and verified later by a visiting extramural scholar who was not blinded to the original interpretation. Any discrepancies between the two readers were resolved through consensus prior to data analysis.

Induction and Maintenance Immunosuppression

All patients received induction therapy consisting of methylprednisolone (15 mg/kg IV) intraoperatively. Until July 30, 2011 Interleukin-2 blockade with daclizumab (1 mg/kg IV) was given either intraoperatively or on postoperative day (POD) 2 and then every 2 weeks for a total of 5 doses. After August 1, 2011, basiliximab (10 or 20 mg IV) was used instead of daclizumab on PODs 1 and 5. After July 1, 2012, rabbit anti-thymocyte globulin (1.5 mg/kg/IV daily x 5 days) was used instead of basiliximab. Plasmapheresis (1.5 volume exchange) followed by intravenous immunoglobulin (IVIG) (2 g/kg) were given per protocol intraoperatively, typically in the setting of pre-transplant IgG+ DSA (MFI >1000) or at the discretion of

the attending transplant cardiologist. Post-operatively, all patients received maintenance immunosuppressive therapy consisting of a calcineurin inhibitor (cyclosporine or tacrolimus), mycophenolate mofetil, and methylprednisolone or prednisone, which was tapered over the first post-transplant year. Cyclosporine and tacrolimus dosing were modulated to achieve target troughs of 300-350 or 10-12 g/dl, respectively, for the first 3 post-transplant months, after which doses were adjusted downward sequentially to goal troughs of 200-250 g/dl at 12 months post-transplant for cyclosporine or 6-8 g/dl by 6 months post-transplant for tacrolimus. Select patients were transitioned to sirolimus at various time points at least 6 months post-transplant and were dosed to achieve a target trough of 6-8 g/dl and continued on reduced-dose cyclosporine or tacrolimus adjusted to achieve target troughs of 100-150 or 2-4 g/dl, respectively. Patients with persistent DSA following cardiac transplantation continued to receive monthly IVIG postoperatively at the discretion of the attending transplant cardiologist.

DSA Assay and C1q Virtual Crossmatch Avoidance Strategy

HLA Class 1 and 2 antibodies were assessed by the IgG and C1q single antigen bead assays as described previously.[14, 15] All patients had a virtual crossmatch (VXM) performed on their most recent calculated panel reactive antibody (cPRA) sample at the time of donor offer. While each donor offer was considered individually based on number of antibody present, mean fluorescence intensity (MFI), overall organ quality, and clinical status of the patient, from an immunological standpoint, in general, organs that caused a C1q+ VXM were typically rejected while organs that were VXM positive by IgG (IgG+) only were typically accepted. DSA were then again assayed on the day of transplant (at the time of flow cyotometry +/- cytotoxic crossmatching), per routine scheduling (POD #14, POD #28, then monthly until 6 post-transplant months, then annually), and during episodes of suspected ACR or AMR at the discretion of the attending cardiologist.

Rejection Surveillance Protocol

Immunohistochemical staining (C3d, C4d, CD68) was performed at the discretion of the cardiac pathologist or at the request of the transplant cardiologist. Peroxidase staining was not performed. Biopsy frequency varied by age, but typically patients would undergo EMB weekly for the first post-HT month, biweekly for the 2nd month, monthly until the 6th post-transplant month, and every 3 months through the second post-transplant year. The majority of patients had EMBs at least twice yearly

thereafter with a small cohort of patients biopsied yearly in the setting of at least moderate tricuspid valve regurgitation and/or poor vascular access. Additional biopsies were performed when rejection was suspected.

Hemodynamic Surveillance

Systolic function was assayed qualitatively (normal, mildly reduced, moderately reduced, severely reduced) by echocardiography, which was performed on the same day as EMB. Starting in 2012, routine hemodynamic measurements were performed at the time of all endomyocardial biopsies. Prior to this, hemodynamic measurements were recorded during most EMBs at the discretion of the interventional cardiologist or the request of the HT cardiologist.

Analysis

The primary outcome studied was the presence or absence of pAMR2 or greater using current ISHLT schema.[6] The total number of EMBs positive for ≥pAMR2 were analyzed as were the total number of ≥pAMR2 episodes, defined as a positive ≥pAMR2 biopsy subsequent to a normal biopsy (i.e. consecutive biopsies positive for ≥pAMR2 were considered as part of a single episode). Secondary outcomes included mean right atrial, pulmonary arterial, and pulmonary capillary wedge pressure (PCWP) measurements, change in either left or right atrial filling pressure from prior catheterization defined as an increase of ≥3 mmHg in atrial filling pressure by direct measurement, mean PCWP/right atrial pressure ratio, and graft dysfunction, defined as a qualitative decrement in systolic function by echocardiogram. The presence or absence of DSA at transplant, including the MFI (using the highest value if present on multiple beads) of the strongest DSA by both the IgG and C1q methods, were also analyzed as risk factors for pAMR development. For DQ antibodies, when alpha typing was reported, DSA were identified by both their DQA1* and DQB1* loci and the highest MFI (if present on multiple beads) used in the analysis. When DQA1* typing was not available, by convention, DQ antibodies were identified by the DQB1* locus only. Changes in IgG and C1q DSA profile were also ascertained at the time of pAMR diagnosis. All biopsies were examined for ACR and the histologic features of AMR. Immunohistochemical staining was performed at the request of the on-call cardiologist or if if there was histological suspicion of at least pAMR1. Per protocol all emergent biopsies and those in follow-up for

prior pAMR positive biopsies were also stained for immunohistochemical findings. For the purposes of analysis, no distinction was made between pAMR1 and pAMR0.

Additional outcomes studied were freedom from ≥pAMR2 and overall post-transplant survival in patients (dichotimized by those with and those without a history of ≥pAMR2), which were depicted with Kaplan-Meier curves. Patients who died without ≥pAMR2 were censored event-free at the time of death. Patients who did not die or have ≥pAMR2 were censored event-free at end of the data collection period. Comparison of HT survival between those with and without ≥pAMR2 groups was performed using the log-rank test. Univariate Cox regression analysis was used to identify risk factors for the development of ≥pAMR2 using all variables with a p value less than 0.1. However, following the methodology employed by Everitt et al., comparisons between patients who did and did not develop ≥pAMR2 were also conducted using Wilcoxon's rank-sum test or Fisher's exact test, as appropriate, which we felt to be acceptable because the number of events was small and mainly occurred during the first HT year, and almost all patients had at least 1 year of follow-up. For continuous data that was normally distributed, comparisons between pAMR+ and pAMR- negative patients were conducted using unpaired t-tests. Data were presented as median (quartile 1-quartile 3), mean ± standard deviation, or count (%).

Data were collected and stored in RedCAP (Version 6.9.7), a web-based application designed to support data capture for research studies.[16] Statistics were performed using Microsoft Excel (Version 14.4.8), and Stata (Version 12.1, STATA Corp.) was used for time-to-event analysis. This study was approved by the Stanford University Institutional Review Board.

Results

Patient Characteristics

Baseline demographics and clinical characteristics of those with and without ≥pAMR2 are reported in Table 1. A total of 102 patients were transplanted during the study period. Three combined heart-liver transplants were excluded. Of the remainder, 96 (97%) patients had at least one EMB and were included in the analysis.

The median age at transplant was 7 (Q1-Q3 1, 15) years. Fifty-four patients (56%) had a pre-transplant diagnosis of cardiomyopathy and 40 (42%) had a pre-transplant history of congenital heart disease (CHD). Thirty-eight (40%) patients were female. There were 316 (median 3.1, Q1-Q3 1.9, 4.6) total follow-up years.

Incidence of AMR

During the study period, 1513 EMBs were performed, of which 1055 (70%) were examined for both the histological and immunohistochemical features of pAMR and 458 (30%) were examined only histologically after the histological features of pAMR were noted to be absent. Thirteen (0.09%) of the total number of biopsies, 3% of those that were evaluated both histologically and immunohistochemically, were positive for pAMR2. There were 10 discrete pAMR2 episodes in 7 (7%) patients, 9 (90%) of which were discovered on routine surveillance biopsies, and one of which was associated with grade 3B/3R rejection. Seven (70%) episodes in 6 patients occurred in the first post-transplant year with a median time to first pAMR2 episode of 16 (Q1-Q3 7,159) days. Three (30%) episodes in 3 patients occurred after the first post-transplant year, 2 (67%) of which were in patients with a history of early pAMR. There were no cases of pAMR3. Freedom from ≥pAMR2 is depicted in Figure 1.

Hemodynamics

No pAMR episodes were associated with systolic dysfunction by echocardiogram. Of 1513 catheterizations, 1351 (89%) included a right atrial pressure (RAP) measurement, and 1325 (88%) included a PCWP measurement. All pAMR2 biopsies included RAP and PCWP pressure measurements, and 11/13 (85%) included a mean pulmonary arterial pressure measurement. Only 1 of 10 (10%) pAMR episodes was associated with new-onset diastolic dysfunction as defined by an increase of ≥3 mmHg in atrial filling pressure by direct measurement. However, mean right atrial (12 ± 6 vs. 7 ± 4, $p<0.001$), pulmonary capillary wedge (16 ± 5 vs. 11 ± 5, $p<0.001$), and pulmonary arterial (22 ± 3 vs. 18 ± 5, $p=0.043$) pressures were significantly higher when ≥pAMR2 was present than when it was not. (Table 2) These differences remained significant for both the right atrial and pulmonary capillary wedge pressures when the 9 ≥pAMR2 biopsies that occurred in the first 3 post-HT months were compared to hemodynamics obtained during the same time period when ≥pAMR2 was absent (14 ± 5 mm Hg vs. 8 ± 4 mmHg, $p<0.001$ and 18 ± 4 vs. 13 ±5, $p<0.001$, respectively).

Risk Factors for The Development of Early & Late AMR

Age, sex, and race were not associated with pAMR (p= 0.16-0.79). Forty-one (43%) transplanted patients were bridged with a ventricular assist device (VAD), none of whom developed pAMR (p=0.02). pAMR was more common in those with a history of CHD (6/7, 86%, p=0.02). In univariate Cox regression analysis, both CHD (HR: 9.9, [95% CI = 1.2-83], p=0.034) and absence of VAD (HR: $8.3x10^{-17}$ [95% CI = 0], p=0.0036) were significantly associated with ≥pAMR2 development.

Thirty-one (32%) patients had IgG+ DSA at the time of transplant, including 6 of the 7 (86%) who developed pAMR (p=0.004). In the subgroup of those with early pAMR, IgG+ DSA at transplant were present in all patients (6/6, 100%), versus 25/65 (38%) who did not (p=0.0001). Patients with pAMR also had an increased median number of IgG+ DSA at transplant (3, Q1-Q3: 2, 5 vs. 0, Q1-Q3: 0,1 p=0.005), though the median MFI of the strongest IgG+ DSA was not significantly different between those with and without pAMR. (3156, Q1-Q3: 2477, 5429 vs. 2215, Q1-Q3: 1688, 3793, p= 0.09) Seventeen (18%) patients had C1q+ DSA discovered at transplant at the time of retrospective crossmatching. This was not significantly different between groups (2/7 vs. 15/89 p=0.6). (Table 3)

At the time of first pAMR diagnosis, data was available for 6 patients, all of whom had IgG+ DSA and 5 of whom (83%) had C1q+ DSA. In 4 cases, C1q+ DSA present at pAMR diagnosis were of different specificities than those present by either the IgG or C1q methods at transplant. Two patients had IgG+ and C1q+ DSA at pAMR diagnosis that had previously been only IgG+ at transplant. One case of pAMR occurred 5 days after transplant in a patient with IgG+/C1q+ DSA at the time of transplant for whom no follow-up sample at the time of pAMR diagnosis was obtained. Details regarding antibody profile at transplant and pAMR diagnosis are shown in in Table 4.

Outcomes Following AMR Diagnosis

All cases of pAMR resolved. Treatments included IVIG in 6, plasmapheresis and IVIG in 2, and bortezomib/rituximab/IVIG/plasmapheresis in 1. One case of late pAMR self-resolved on the first follow-up biopsy without treatment. During the follow-up period, 3/7 (43%) patients with pAMR died versus 13 of 89 (15%) without AMR. The median time from first pAMR episode to death was 369 (min-max 138-836) days. Although there was no difference in post-transplant survival in those with and without pAMR2 (p=0.15), there was a trend towards reduced post-transplant survival in the early pAMR2 group

(p=0.057) (Figure 2). Of the 3 patients with pAMR2 who died, 1 died of known CAV, 1 had sudden cardiac death secondary to presumed CAV, and 1 died of sepsis in the setting of chronic diastolic dysfunction and dialysis-dependent renal failure.

Discussion

We found that pAMR2 affects 7% of pediatric HT recipients in the first 5 post-transplant years at our center. There were no cases of pAMR3 during the same time period. A history of CHD was associated with pAMR2 development, but no patient who was bridged to transplantation with VAD developed pAMR. The presence of DSA at the time of transplant was associated with early pAMR2. Most patients had new C1q+ DSA at the time of pAMR2 diagnosis. Atrial filling pressures were higher when pAMR2 was present than when it was not, though atrial pressures did not acutely change with pAMR diagnosis. Despite biopsy resolution of pAMR2, patients with pAMR2 were at high risk of death within approximately 2 years of diagnosis.

The reported prevalence of AMR in children varies, likely due to changes in AMR incidence over time, institutional practice including the frequency of routine EMB screening, and differences in the way AMR has been previously defined.[4, 9, 11, 12, 17] Using the Pediatric Heart Transplant Study database, Thrush et al. found that 11% of pediatric patients experienced at least one episode of AMR over 5 years. However, they used a more inclusive definition, including patients who received immunotherapy against antibody production but did not meet the 2011 ISHLT criteria.[4] Everitt et al. reported pAMR2 or higher occurring in 59% of patients and 18% of biopsies, including 12% of patients experiencing pAMR3, however their study examined an earlier patient cohort over a longer follow-up period.[11] Using an updated cohort of pediatric HT patients followed between 2009-2013, Ware et al. reported pAMR occurring in 21% of patients and 11% of biopsies.[12] Our finding that pAMR2 occurred in 7% of patients was similar to that of Clerkin et al., who using the 2013 ISHLT pAMR criteria, reported a 10% incidence of AMR over >10 years follow-up in adults, although it is important to note that the majority of AMR positive biopsies in that series demonstrated pAMR1.[9]

We found the presence of preformed IgG+ DSA at transplant to be associated with pAMR development, as has been reported by others; however, we also observed that 86% patients who developed pAMR2 had new C1q+ DSA at the time of diagnosis, supporting the hypothesis that complement fixation plays an important role in the pathology of pAMR,.[12, 14, 18-20] It is possible that the lower incidence of AMR in

this study is the result of our institutional policy to ensure a C1q-DSA negative virtual crossmatch at the time of donor acceptance, though further study is warranted.

Because of the overall low incidence of pAMR2 in our study, clinical risk factors for pAMR2 development were difficult to establish in this series. Nevertheless, we found that a history of CHD and not having a history of VAD support were more common in patients who developed pAMR2. CHD history has been established previously as a risk factor for AMR, likely due to increased HLA sensitization from exposure to blood transfusions and/or homograft tissue.[4, 21] Conversely, whereas a history of VAD support has been shown to be a risk factor for AMR development in adults, the relationship is less clear in children.[4, 22, 23] Our finding that VAD-supported patients were less likely to develop pAMR2 is most probably related to the disproportionate use of VADs in children with cardiomyopathy versus those with CHD rather than to any protective effect from VAD use.

The present study also found that early pAMR2 development trended towards reduced survival and was associated with higher filling pressures, though it is important to note that pAMR diagnosis was not associated with an acute change in filling pressures. It is also important to acknowledge that the overall incidence of pAMR in this study was very low, limiting the study's ability to draw conclusions as to the effect of pAMR diagnosis on survival. It is quite possible that the trend towards reduced survival in those with a history of pAMR2 observed in this study was the result of coincident factors present in patients with higher risk for pAMR development (e.g. sensitization, congenital heart disease, etc.). Nevertheless, the findings of this study are consistent with those of Clerkin et al., who reported decreased post-AMR survival and accelerated development of CAV in patients with late AMR, 84% of whom were classified as pAMR1.[9] Given the infrequency of pAMR (<1% of biopsies), however, a targeted approach to surveillance in patients at risk for pAMR may be prudent.

Despite a lack of guidelines or even a strong consensus about what constitutes best practice, it has been our institutional approach to treat pAMR2, even in the absence of altered hemodynamics or concomitant ACR. In the present study, all but 1 pAMR2 episode was treated with either IVIG alone, a combination of IVIG and plamsmapheresis, or, in 1 case, IVIG/plasmapheresis/rituximab/bortezomib. The use of IVIG +/- plasmapheresis and/or rituximab is consistent with practices reported by other centers, with evidence supporting the use of bortezomib limited to smaller series.[4, 25, 26] It is important to

note, however, that in the present study all episodes of pAMR2 resolved, including one episode that was not treated, but that resolution of pAMR did not appear to abrogate mortality risk. Although the relationship between AMR and increased risk for poor outcomes even after treatment is well-established, our finding that a single acute pAMR2 episode may herald graft loss, perhaps due to chronic diastolic dysfunction and/or accelerated CAV development, highlights the importance of a pAMR prevention strategy at the time of transplantation. [4, 8, 9, 11, 17]

This study is limited by its retrospective nature and the potential for error inherent upon chart review, as well as a lack of standardization of immunosuppressive regimens over time and individual variability in immunomodulatory therapies. Furthermore, our quantitative interpretation of antibody strength was assumed by MFI, which due to the possibility of the prozone effect and other factors is not a true surrogate for antibody titer. It is also limited by our inability to distinguish pAMR1i from pAMR0. Because immunohistochemical staining is not performed universally on all biopsies, the true incidence of pAMR1i could not be ascertained and the outcomes following pAMR1i could not be determined. It is also important to note that the false positive rate for pAMR may be higher in the first two postoperative weeks when the majority of cases were detected.[6] Moreover, variations in center practice with regard to routine EMB screening limit the generalizability of the results of this single center study.[27] Because the majority of pAMR episodes in this study were detected during routine screening, centers that perform fewer EMBs in asymptomatic patients may find a lower incidence of pAMR as a result of missed events. Lastly, because of the low incidence of pAMR, our ability to identify risk factors for pAMR development was limited. Nevertheless, this study adds valuable information to the current understanding of pAMR using standardized definitions, long-term follow-up, robust DSA data, frequent hemodynamic surveillance, and relatively large sample size.

Acknowledgements and Disclosures

The authors have no conflicts of interest or financial relationships to disclose.

References

1. Hammond EH, Yowell RL, Nunoda S, Menlove RL, Renlund DG, Bristow MR, et al. Vascular (humoral) rejection in heart transplantation: Pathologic observations and clinical implications. *J Heart Transplant*. 1989;8:430-443
2. Patel JK. Early and late amr in heart transplantation-distinct entities? *J Heart Lung Transplant*. 2016;35:1055-1056
3. Coutance G, Ouldamar S, Rouvier P, Saheb S, Suberbielle C, Brechot N, et al. Late antibody-mediated rejection after heart transplantation: Mortality, graft function, and fulminant cardiac allograft vasculopathy. *J Heart Lung Transplant*. 2015;34:1050-1057
4. Thrush PT, Pahl E, Naftel DC, Pruitt E, Everitt MD, Missler H, et al. A multi-institutional evaluation of antibody-mediated rejection utilizing the pediatric heart transplant study database: Incidence, therapies and outcomes. *J Heart Lung Transplant*. 2016;35:1497-1504
5. Kucirka LM, Maleszewski JJ, Segev DL, Halushka MK. Survey of north american pathologist practices regarding antibody-mediated rejection in cardiac transplant biopsies. *Cardiovasc Pathol*. 2011;20:132-138
6. Berry GJ, Angelini A, Burke MM, Bruneval P, Fishbein MC, Hammond E, et al. The ishlt working formulation for pathologic diagnosis of antibody-mediated rejection in heart transplantation: Evolution and current status (2005-2011). *J Heart Lung Transplant*. 2011;30:601-611
7. Berry B, Rodriiguez-Jimenez TM. International trends in health science librarianship. Part 5 latin america and the caribbean. *Health Info Libr J*. 2013;30:76-82
8. Kfoury AG, Renlund DG, Snow GL, Stehlik J, Folsom JW, Fisher PW, et al. A clinical correlation study of severity of antibody-mediated rejection and cardiovascular mortality in heart transplantation. *J Heart Lung Transplant*. 2009;28:51-57
9. Clerkin KJ, Restaino SW, Zorn E, Vasilescu ER, Marboe CC, Mancini DM. The effect of timing and graft dysfunction on survival and cardiac allograft vasculopathy in antibody-mediated rejection. *J Heart Lung Transplant*. 2016;35:1059-1066
10. Kfoury AG, Stehlik J, Renlund DG, Snow G, Seaman JT, Gilbert EM, et al. Impact of repetitive episodes of antibody-mediated or cellular rejection on cardiovascular mortality in cardiac transplant recipients: Defining rejection patterns. *J Heart Lung Transplant*. 2006;25:1277-1282
11. Everitt MD, Hammond ME, Snow GL, Stehlik J, Revelo MP, Miller DV, et al. Biopsy-diagnosed antibody-mediated rejection based on the proposed international society for heart and lung transplantation working formulation is associated with adverse cardiovascular outcomes after pediatric heart transplant. *J Heart Lung Transplant*. 2012;31:686-693

12. Ware AL, Malmberg E, Delgado JC, Hammond ME, Miller DV, Stehlik J, et al. The use of circulating donor specific antibody to predict biopsy diagnosis of antibody-mediated rejection and to provide prognostic value after heart transplantation in children. *J Heart Lung Transplant*. 2016;35:179-185
13. Das BB, Lacelle C, Zhang S, Gao A, Fixler D. Complement (c1q) binding de novo donor specific antibodies and cardiac-allograft vasculopathy in pediatric heart transplant recipients. *Transplantation*. 2017
14. Chin C, Chen G, Sequeria F, Berry G, Siehr S, Bernstein D, et al. Clinical usefulness of a novel c1q assay to detect immunoglobulin g antibodies capable of fixing complement in sensitized pediatric heart transplant patients. *J Heart Lung Transplant*. 2011;30:158-163
15. Chen G, Tyan DB. C1q assay for the detection of complement fixing antibody to hla antigens. *Methods Mol Biol*. 2013;1034:305-311
16. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (redcap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42:377-381
17. Casarez TW, Perens G, Williams RJ, Kutay E, Fishbein MC, Reed EF, et al. Humoral rejection in pediatric orthotopic heart transplantation. *J Heart Lung Transplant*. 2007;26:114-119
18. Peng DM, Law YM, Kemna MS, Warner P, Nelson K, Boucek RJ. Donor-specific antibodies: Can they predict c4d deposition in pediatric heart recipients? *Pediatr Transplant*. 2013;17:429-435
19. Farrero Torres M, Pando MJ, Luo C, Luikart H, Valantine H, Khush K. The role of complement-fixing donor specific antibodies identified by a c1q assay after heart transplantation. *Clin Transplant*. 2017
20. Mangiola M, Marrari M, Feingold B, Zeevi A. Significance of anti-hla antibodies on adult and pediatric heart allograft outcomes. *Front Immunol*. 2017;8:4
21. Shaddy RE, Thompson DD, Osborne KA, Hawkins JA, Fuller TC. Persistence of human leukocyte antigen (hla) antibodies after one year in children receiving cryopreserved valved allografts. *Am J Cardiol*. 1997;80:358-359
22. Nair N, Ball T, Uber PA, Mehra MR. Current and future challenges in therapy for antibody-mediated rejection. *J Heart Lung Transplant*. 2011;30:612-617
23. Michaels PJ, Espejo ML, Kobashigawa J, Alejos JC, Burch C, Takemoto S, et al. Humoral rejection in cardiac transplantation: Risk factors, hemodynamic consequences and relationship to transplant coronary artery disease. *J Heart Lung Transplant*. 2003;22:58-69

24. Lunze FI, Colan SD, Gauvreau K, Chen MH, Perez-Atayde AR, Blume ED, et al. Cardiac allograft function during the first year after transplantation in rejection-free children and young adults. *Circ Cardiovasc Imaging*. 2012;5:756-764

25. Chih S, Tinckam KJ, Ross HJ. A survey of current practice for antibody-mediated rejection in heart transplantation. *Am J Transplant*. 2013;13:1069-1074

26. Morrow WR, Frazier EA, Mahle WT, Harville TO, Pye SE, Knecht KR, et al. Rapid reduction in donor-specific anti-human leukocyte antigen antibodies and reversal of antibody-mediated rejection with bortezomib in pediatric heart transplant patients. *Transplantation*. 2012;93:319-324

27. Godown J, Harris MT, Burger J, Dodd DA. Variation in the use of surveillance endomyocardial biopsy among pediatric heart transplant centers over time. *Pediatr Transplant*. 2015;19:612-617

Table 1: Baseline Demographics and Clinical Characteristics (n=96)

	All Patients	pAMR+	pAMR-	*P*-Value
N (% of total)	96	7 (6%)	89 (93%)	
Age at transplant, years	7 (1,15)	7 (2, 12)	8 (1,15)	0.79
Sex, Female	38 (40%)	1 (14%)	37 (42%)	0.24
Race/Ethnicity				
White	44 (46%)	5 (71%)	39 (44%)	0.16
African American	6 (6%)	1 (14%)	5 (6%)	
Hispanic/Latino	35 (36%)	1 (14%)	34 (38%)	
Asian/Hawaiian/Pacific Islander	8 (8%)	0 (0%)	8 (9%)	
Other	3 (3%)	0 (0%0	3 (3%)	
Pre-Transplant Diagnosis				**0.02***
Cardiomyopathy	54 (56%)	1 (14%)	53 (60%)	
CHD	40 (42%)	6 (86%)	34 (38%)	
Retransplantation	2 (2%)	0 (0%)	2 (2%)	

Pre-Transplant VAD	41 (43%)	0 (0%)	41 (100%)	**0.02**
Duration of Follow-Up (Years)	3.1 (1.9, 4.6)	4.7 (1.4, 5.2)	3 (1.9, 4.5)	0.56

Data presented as Median (quartile 1 – quartile 3) or count (% of column)

*Retransplants not included in this analysis.

CHD; Congenital Heart Disease, pAMR; Pathological Antibody-Mediated Rejection, VAD; Ventricular Assist Device

Table 2: Hemodynamic measurements obtained during right heart catheterization in biopsies with and without pAMR2.

	All Patients	**pAMR2+**	**pAMR2-**	***P*-Value**
Mean RA pressure, n=1351	7 (+/-4)	12 (+/- 6)	7 (+/- 4)	**<0.001**
Mean PCWP, n=1325	11 (+/- 5)	16 (+/-5)	11(+/-5)	**<0.001**
Mean PA pressure, n=1275	18 (+/-5)	22 (+/- 3)	18 (+/-5)	**0.043**
Mean RA pressure/PCWP ratio, n=1314	2 (+/- 1)	2 (+/- 0.5)	2 (+/- 1)	0.20

Data are normally distributed and are presented as mean (+/- standard deviation)

PA, pulmonary artery; PCWP, pulmonary capillary wedge pressure; RA, right atrium

Table 3: DSA and MFI at time of transplant among those who did and did not develop pAMR.

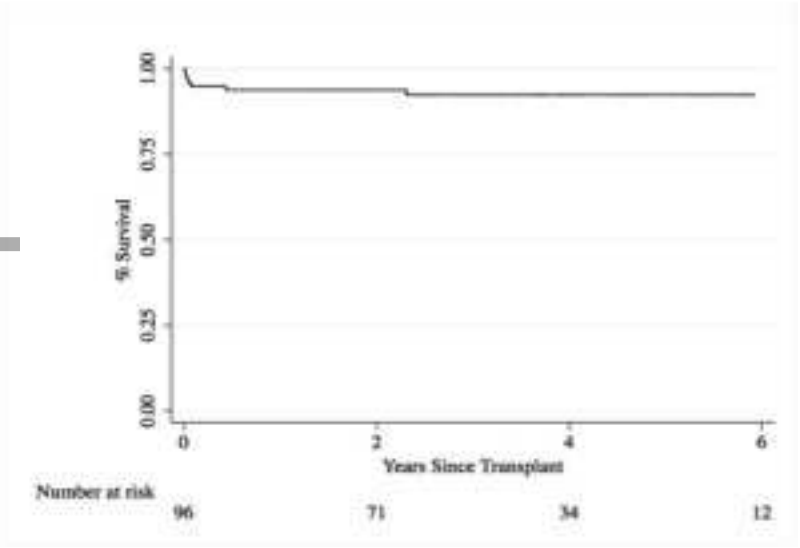
	All Patients	pAMR+	pAMR-	*P*-Value
N (% of total)	96	7 (6%)	89 (93%)	
Any Pre-Formed IgG+ DSA	31 (32%)	6 (86%)	25 (28%)	**0.004**
Number of Different Pre-Formed IgG+ DSA	1 (1,1)	3 (2,5)	0 (0,1)	**0.005**
Highest MFI Pre-Formed IgG+ DSA	2383 (1689, 4282)	3156 (2477, 5429)	2215 (1688, 3793)	0.09
Any Pre-Formed C1q+ DSA	17 (18%)	2 (29%)	15 (17%)	0.6
Number of Different Pre-Formed C1q+ DSA	0 (0,0)	0 (0,1)	0 (0,0)	0.4
Highest MFI Pre-Formed C1q+ DSA	2915 (2046, 3476)	13,807 (2050, 25,564)	2915 (1889, 3476)	0.5

Table 4: DSA present at transplant and during first pAMR episode in pAMR+ patients.

Pt. No.	Retrospective Crossmatch (B/T Cell Flow Cytometry)	IgG+ DSA at Transplant	C1q+ DSA at Transplant	Days to pAMR diagnosis	IgG+ DSA at pAMR Diagnosis	C1q+ DSA at pAMR Diagnosis	Outcome
1	neg/neg	DR17, DQ2 (DQA1*05:01:DQB1*02:01)	None	17	C7, A1, A2, B49, DQ2	A2, B49, B8, C7	Died of sepsis 138 days after diagnosis
2	neg/pos[⌘]	DR4	None	160	DQ7	DQ7	Died suddenly, cause unknown 369 days after diagnosis
3	neg/neg	**B27**[^], C15, **DR15**, DR103, DR51, **DQ5 (DQA1*01:01:DQB1*05:01)**, DQ6	None	27	A2, **B27**, BW4, C15, **DR15**, DR103, DR51, **DQ5**,	**B27**, BW4, **DR15, DQ5 (DQA1*01:01:DQB1*05:01)**	AMR resolved, developed GCAD, died 839 days after diagnosis
4	neg/neg	C2, C7, DR4	None	7	C2, C7, DR4	None	AMR resolved, alive

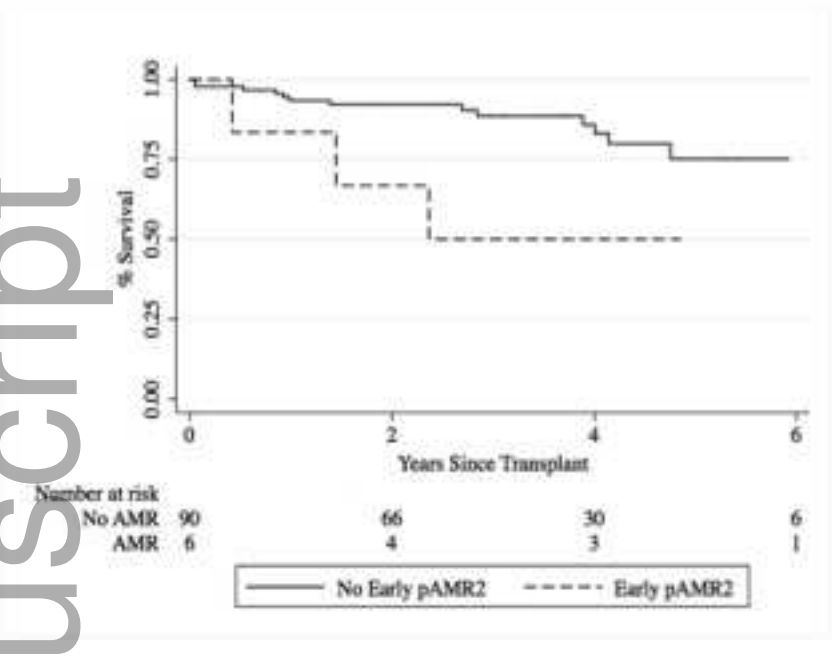
5	neg/neg	None	B75[§]	843	A2	A2, DQ6	AMR resolved, alive
6	pos/neg	DR4, **DQ8**	None	12	B7 DR4 DR15 DR 51 DR53 DQ6 **DQ8**	B7 DR4 DR15 DR51 DR53 DQ6 **DQ8** DQA1*03	AMR resolved, alive
7	pos/pos[*]	A2, A24, DR4,	A2, A24	5	N/A	N/A	AMR resolved, alive

Figure 1: Freedom from pAMR2 development (n=96).



petr_13197_f1.tiff

Figure 2: Freedom from death in patients with and without early pAMR2. (n=96)



petr_13197_f2.tiff