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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

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BACKGROUND: Antibody-mediated rejection (AMR) is a significant cause of mortality after heart transplantation (HT). Although the presence of donor specific antibody (DSA) is a risk factor for developing AMR, serial DSA testing is not widely performed. We aimed to investigate the predictive values and prognostic implications of circulating DSA using endomyocardial biopsy as the gold standard for AMR diagnosis in pediatric recipients of HT.

METHODS: We performed a retrospective study in pediatric recipients of HT followed during the period 2009–2013 with at least 1 biopsy paired with DSA testing. Positive DSA was defined at mean fluorescent intensity (MFI) $\geq$2,000 using single antigen bead testing. Statistical analyses included 2 $\times$ 2 contingency tables, receiver operating characteristic analysis for optimal MFI cutoffs, Spearman correlation of MFI strength to AMR grade, and Kaplan-Meier analysis of event-free survival.

RESULTS: Of 66 children included, 27 (41%) had $\geq$1 DSA positive test. DSA testing had a sensitivity of 92.6%, specificity of 62.2%, positive predictive value of 24.0%, and negative predictive value of 98.5% for biopsy diagnosis of AMR at our institution. There was a statistically significant correlation between higher MFI and higher AMR grade. Patients with positive DSA and AMR had similar survival early after DSA detection but trended toward lower cardiovascular event–free survival later compared with patients without DSA and a negative biopsy.

CONCLUSIONS: The results of DSA testing in this cohort showed excellent sensitivity and negative predictive value for biopsy-diagnosed AMR, suggesting that DSA testing may aid in the non-invasive prediction of AMR absence in HT. The correlation of DSA MFI strength with higher AMR biopsy grade

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and the trend toward differences in longer term cardiovascular outcomes provide evidence for routine DSA monitoring after pediatric HT.
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Heart transplantation (HT) is a lifesaving option for children with end-stage heart failure. Cardiac allograft rejection remains a significant cause of morbidity and mortality after HT.[1] Since 2005, allograft rejection has been classified as cellular rejection or antibody-mediated rejection (AMR).[2] Acute cellular rejection has been well described, but AMR and its implications on clinical practice are less understood.[3] The current diagnostic criteria for AMR are based on endomyocardial biopsy (EMB) showing immunopathologic evidence of C4d or C3d deposition, and histologic evidence of capillary endothelial changes, neutrophil and macrophage infiltration, and interstitial edema.[3] Pathologic AMR (pAMR) has been associated with graft dysfunction, hemodynamic compromise, and poor cardiovascular outcome.[3–8] Identifying patients at risk for the development of AMR and diagnosing AMR before the development of graft dysfunction may lead to improved outcomes after HT.

The presence of donor specific antibody (DSA) has been identified as a risk factor for the development of AMR after HT,[9–11] and it is recommended that testing for DSA be performed when there is biopsy diagnosis of AMR.[3] At the present time, the diagnosis of pAMR requires invasive EMB, which can be associated with procedural complications.[12] We sought to determine the sensitivity, specificity, and predictive value of circulating DSA using the gold standard of biopsy diagnosis of AMR after HT in a cohort of pediatric recipients of HT. Our secondary aim was to examine the prognostic implications of circulating DSA after HT in children with and without the presence of pAMR.

Methods

Design and patient cohort

This study was approved by the institutional review board at the University of Utah Medical Center and the privacy board of Primary Children's Hospital. This was a retrospective review of all recipients $\leq$21 years old at HT followed at our center between January 2009 and September 2013. Patients were included in the analysis if they had at least 1 EMB paired with DSA testing during the study period. EMB results were included if a DSA test was obtained within 3 days of the biopsy. Patients without any EMB paired with DSA testing were excluded from the study cohort. EMB results were excluded if there was no paired DSA result. Outcomes evaluated included all-cause mortality, cardiac death defined as graft-related mortality or retransplant, and a combination of cardiac death and coronary allograft vasculopathy (CAV).

Standardized testing for DSA and AMR

Since 2005, the measurement of DSA has been part of our routine evaluation of pediatric recipients of HT. DSA testing is paired with cardiac biopsy performed within 4 weeks of HT, at 4–6 months after HT, and at year 1 after HT for all HT recipients. DSA testing is repeated in patients with a previously positive DSA; patients with a history of AMR; or patients at risk for developing DSA, defined as patients with an elevated panel reactive antibody (PRA) before transplant, repaired congenital heart disease, mechanical circulatory support, non-compliance with medication, or a history of severe rejection. For DSA testing, sera were screened for anti-human leukocyte antigen (HLA) following the manufacturer's suggested protocol using a panel of up to 100 different color-coded beads each coated with purified single HLA class I and class II antigens (LABScreen Single Antigen Beads; One Lambda, Inc., Canoga Park, CA), using the LABScan 100 flow analyzer (Luminex Corporation, Austin, TX). Interassay variability of semi-quantitative DSA results is $<$20% based on validation by the Histocompatibility and Immunogenetics laboratory of our program. Positivity for DSA at our institution is defined as an antibody to donor HLA detected at mean fluorescent intensity (MFI) $\geq$2,000.

Screening for pAMR is standard at our institution on all EMB specimens obtained. Since 2010, biopsy findings have been graded by a team of 3 cardiac pathologists according to the 2010 International Society for Heart and Lung Transplantation (ISHLT) guidelines.[13] EMB specimens obtained before the 2010 revision were assigned a pAMR grade based on the detailed semi-qualitative scoring of all independent features of AMR in the U.T.A.H. Cardiac Pathology Database as previously described.[4]

Statistical analysis

All analyses were carried out using SAS 9.4 (SAS Institute, Cary, NC). Descriptive statistics including percentage and range are reported. A Pearson correlation between MFI values and pAMR grades was evaluated for class I and class II antibodies. The sensitivity, specificity, and predictive value of DSA testing to predict the presence of pAMR on biopsy was determined using standard 2 $\times$ 2 contingency tables. Positive DSA was defined as any DSA at MFI $\geq$2,000. We assessed the relationship between any evidence of pAMR (1h, 1i, 2, or 3) and the relationship of pAMR 2 or 3 only. Additionally, the sensitivity and specificity of class I and class II MFI levels to predict the presence of any pAMR (grades 1h, 1i, 2, or 3) was evaluated using the above-mentioned pAMR grades and increasing the definition of a positive MFI cutoff by intervals of 100.[9,14] Receiver operating characteristic (ROC) analysis was used to determine the MFI cutoff with optimal sensitivity and specificity for class I and class II antibodies. ROC analysis is used to determine the relationship between specificity and sensitivity with each point on the figure representing the specificity and sensitivity for a given MFI cutoff. The ROC curve visually demonstrates the cutoff with the maximal sensitivity and specificity. A similar analysis was performed to predict the optimal MFI cutoffs for pAMR grade 2 or 3.

For outcomes analysis, we focused on pathologic AMR grades of 2 or 3 as clinically important pathologic findings. This decision was based on previous work at our institution and our practice to treat or optimize baseline immune suppression in all episodes of pAMR 2 and to treat all episodes of pAMR 3.[4] Biopsy evidence of pAMR 1 is not treated at our institution unless the patient has