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Impact of age on incidence and prevalence of moderate-to-severe cellular rejection detected by routine surveillance biopsy in pediatric heart transplantation

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KEYWORDS:
pediatric heart transplantation;
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BACKGROUND: The effect of age at transplant on rejection detected by routine surveillance biopsy (RSB) in pediatric heart transplant (HT) recipients is unknown. We hypothesized there would be low diagnostic yield and decreased prevalence of rejection detected on RSB in infants (age <1 year) when compared with children (age 1 to 9 years) and adolescents (age 10 to 18 years).

METHODS: We utilized Pediatric Heart Transplant Study (PHTS) data from 2010 to 2013 to analyze moderate-to-severe (ISHLT Grade 2R/3R) cellular rejection (MSR) detected only on RSB (RSBMSR).

RESULTS: RSB detected 280 of 343 (81.6%) episodes of MSR. RSBMSR was detected in all age groups even >5 years after HT. Infant RSBMSR had a greater proportion ($p = 0.0025$) occurring >5 years after HT (39.2 vs 18.4 vs 10.8%) and a lower proportion ($p = 0.0009$) occurring in the first year after HT (25.5 vs 60.6 vs 51.7%) compared with children and adolescents, respectively. Freedom from RSBMSR was 87 ± 7% in infants, 76 ± 6% in children and 73 ± 7% in adolescents 4 years after HT. In 1-year survivors who had RSBMSR in the first year after HT, the risk of RSBMSR occurring in Years 2 to 4 was significantly ($p < 0.0001$) greater than patients without RSBMSR in the first year (hazard ratio 21.28, 95% confidence interval 10.87 to 41.66), regardless of recipient age.

CONCLUSIONS: RSBMSR exists in all age groups after pediatric HT with long-term follow-up. The prevalence in infant recipients is highest >5 years after HT. Those with RSBMSR in the first year after HT are at a high risk for recurrent rejection regardless of age at HT.

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One of the major complications after solid-organ transplant is graft rejection. The bulk of management decisions revolve around rejection prevention, monitoring and treatment. Fortunately, the incidence of acute cellular rejection (ACR) after pediatric heart transplantation (HT) has

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decreased in recent years secondary to advancements in immunosuppression, identification and human leukocyte antigen (HLA) matching.[1,2] The first year after transplant remains the time of greatest risk, with decreasing incidence in later years.[1–4] Age at transplant plays a role as well, with the lowest risk reported in infant recipients, whereas older recipients carry a higher risk of rejection.[1,2,5]

Diagnosing rejection can be relatively straightforward in a symptomatic patient. Acute graft dysfunction from ACR often presents with signs and symptoms of heart failure, such as dyspnea, tachycardia and peripheral edema. Conversely, ACR may be present in a completely asymptomatic patient, making early identification extremely difficult. Non-invasive imaging has shown promise in differentiating rejection from non-rejection, but results lack the degree of consistency needed to replace endomyocardial biopsy (EMB) as the diagnostic standard in ACR.[6,7]

Beyond obtaining biopsies for episodes of symptomatic rejection, multiple routine surveillance biopsies (RSB) are performed for ACR monitoring in asymptomatic patients, especially during the first year post-transplant. In 2000, a large, single-center study reported the incidence of moderate-to-severe ACR (i.e., ISHLT Grade 2R/3R) diagnosed on RSB as 18% in the first year, 12% between Years 1 to 5 and 2.9% at >5 years after HT.[8] In Years 1 to 5, infants and those without rejection in the first year after HT showed a decreased incidence of RSBMSR, but the data did not reach statistical significance. Current data are lacking on the diagnostic yield of RSB, including whether or not the age at transplant and time from transplant-dependent prevalence follows the same pattern as previous reports. For this study, we proposed 2 hypotheses: (1) routine surveillance biopsy will have a low diagnostic yield compared with symptomatic indications for biopsy; and (2) RSBMSR will show a decreasing prevalence over time, with the lowest prevalence seen in infants compared with children and adolescents.

Methods

Data were acquired from the Pediatric Heart Transplant Study Group (PHTS). This prospectively collected database is managed at the University of Alabama at Birmingham. PHTS membership currently includes 52 centers across 3 continents.

This retrospective database review included all enrolled individuals ≤18 years of age with at least 1 episode of moderate-to-severe rejection (MSR) between 2010 and 2013. This time frame was chosen because the database began collecting data on biopsy indication in 2010. Rejection was defined as a biopsy-driven clinical episode that resulted in an augmentation of immunosuppression. Only MSR rejection (ISHLT 2R/3R) was included in the study to eliminate the interpretive variability surrounding Grade 1R classification. Patients who received a multiple-organ transplant were excluded from the analysis. Recipient age groups were defined as infants (age <1 year), children (age 1 to 9 years) and adolescents (age 10 to 18 years). Post-transplant time periods included the first year, Years 2 to 5 and >5 years.

Data obtained from PHTS included date of birth, gender, race, date of transplant, panel-reactive antibody (PRA) percentage at transplant, date of rejection episode, baseline immunosuppression at transplant, biopsy score, indication for biopsy, rejection therapy and degree of hemodynamic compromise at the time of rejection.

Statistical analysis

All analyses were performed at the School of Medicine, Washington University of St. Louis. Study-specific variables were extracted from the PHTS limited data set to create a unique data set for analysis. Proportions were estimated with contingency tables and tested for differences with Pearson's chi-square test. Survival and time to rejection were estimated with product-limit survival curves. Group hazard rates were estimated and compared with Cox proportional hazard regression. All analyses were performed with SAS version 9.4 (SAS Institute, Inc., Cary, NC).

Results

A total of 797 rejection episodes were reported to PHTS from 2010 to 2013 (Figure 1). The indication for biopsy was missing for 101 rejection episodes, requiring exclusion from analysis. Another 68 were excluded for failing to produce a change in immunosuppression. Of the remaining 628 rejection episodes, 476 (76%) listed "routine protocol" as the biopsy indication. One additional episode listed the biopsy indication as "routine protocol" and "symptoms." Additional biopsy indications included "symptoms" in 106 (16.9%) and "objective evidence of graft dysfunction" in 45 (7.1%). Patients' demographics based on biopsy indication are presented in Table 1.

Prevalence of MSR diagnosed on routine surveillance biopsy

The data set contained 343 episodes of MSR. Routine biopsy was responsible for identifying 280 (81.6%) episodes of MSR. RSBMSR was detected in all age groups during the 3 study time periods after HT, and was the primary indication for biopsy for all patients (Table 2). The greatest prevalence of RSBMSR in infants was found >5 years after HT (39.2%), with the lowest prevalence occurring in the first year after HT (25.5%). The opposite pattern was seen in children and adolescents. Most RSBMSR was found in the first year after HT (children 60.6%, adolescents 51.7%), with a steady reduction over time to the lowest prevalence found beyond Year 5. There was a significant difference between the prevalence of RSBMSR during the 3 time periods when analyzed by age group. The total percentage of MSR diagnosed by RSB did not differ significantly among age groups (infants 89.5%, children 82%, adolescents 78.4%).

Incidence of MSR diagnosed on routine surveillance biopsy

Freedom from RSBMSR was significantly ($p < 0.001$) greater in infants compared with children and adolescents (87 ± 7% in infants, 76 ± 6% in children, 73 ± 7% in adolescents) at 4 years post-HT (Figure 2). The hazard of RSBMSR was significantly lower ($p < 0.0001$) in infants

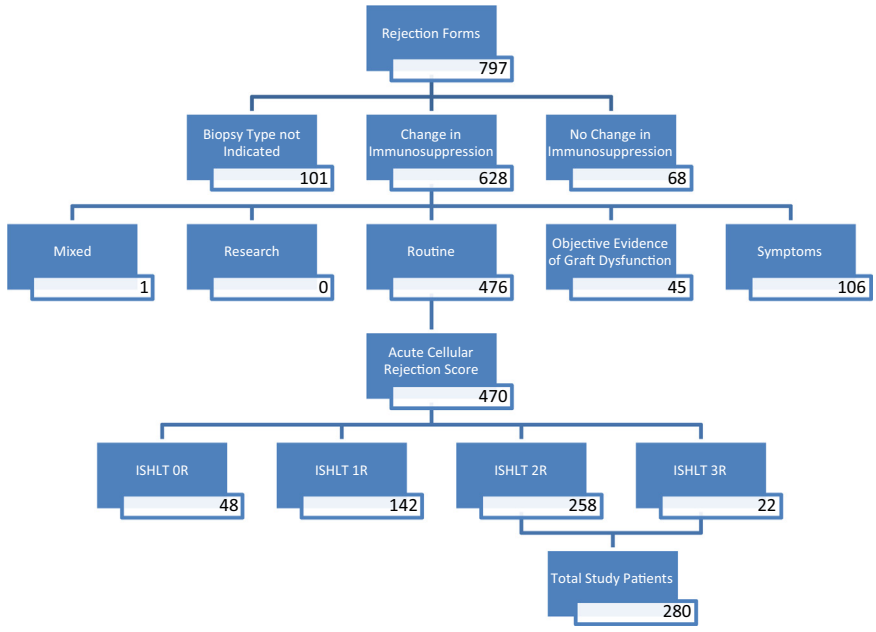

Figure 1 Patient inclusion algorithm.

compared with children (hazard ratio [HR] 3.28, 95% confidence interval [CI] 2.02 to 5.32) and adolescents (HR 3.42, 95% CI 2.12 to 5.36) (Figure 3). We also evaluated the association of recurrent RSBMSR in those who survived an episode in the first year after HT (Figure 4). The risk of subsequent RSBMSR in Years 2 to 4 was significantly ($p < 0.0001$) greater in patients with RSBMSR in the first year after HT compared to those without RSBMSR in the first year after HT (HR 21.28, 95% CI 10.87 to 41.66). Freedom from subsequent RSBMSR was 8.7% 3 years after HT for those with a first-year episode surviving beyond the first year. Absence of first year RSBMSR was associated with 91% freedom from MSR over the next 3 years for those surviving beyond the first year. This effect was seen regardless of the patient's age at transplant. No significant confounders were identified when further evaluating patients with recurrent RSBMSR compared to patients without recurrent RSBMSR. Patients with recurrent RSBMSR were mostly listed as white race and not on prednisone at the time of the second rejection episode. Gender, baseline immunosuppression and PRA at the time of transplant were not different between the 2 groups.

Discussion

EMB is the mainstay of rejection monitoring after HT. Many pediatric rejection surveillance protocols are front loaded with frequent early surveillance biopsies due to a historically higher incidence of rejection in the first year.[1–3,8] However, data exist to the contrary, particularly in infant HT recipients. In 1994, Zales et al reported negative RSBs in all infants ($n = 14$) without clinical signs of rejection, whereas 8.4% of RSBs were positive for rejection (ISHLT Grade ≥ 2) in older children ($n = 21$).[9] The mean follow-up time for the infant group and older child group was 25 months and 21.5 months, respectively. Levi et al showed only a 0.36% incidence of EMB-positive rejection (ISHLT Grade $\geq 1B$) in asymptomatic patients on tacrolimus therapy (770 biopsies) during the first year after transplant, and no asymptomatic patient had a positive biopsy at >1 year after transplant.[10] In contrast, other reports suggest RSB continues to detect late episodes of MSR. Kuhn et al reported an 8% to 10% annual incidence of moderate rejection up to 11 years after transplantation in a study including 269 children with a mean follow-up time of 5 years.[11]

Table 1 Patients' Demographics by Biopsy Indication

	Routine Protocol	Symptoms or OEGD	*p*-value
Female gender	50.9%	54.6%	0.70
Black race	24.9%	27.7%	0.77
White race	68.5%	66.7%	0.84
Absence of hemodynamic compromise	89.1%	24.2%	<0.05
Baseline immunosuppression			
Azathioprine	9.7%	12.1%	0.67
MMF	78.2%	66.7%	0.16
Prednisone	50.3%	30.3%	0.04
Tacrolimus	86.7%	87.9%	0.85
PRA at transplant (median)	0%	0%	0.93

MMF, mycophenolate mofetil; PRA, panel-reactive antibody; OEGD, objective evidence of graft dysfunction.

Table 2 Prevalence of RSBMSR by Age and Time After HT

	Year 1 post-HT	Years 2 to 5 post-HT	>5 years post-HT	Number (%) of all reported 2R/3R rejections
RSBMSR				
Infants ($n = 51$)	13 (25.5%)	18 (35.3%)	20 (39.2%)	51 of 57 (89.5%)
Children ($n = 109$)	66 (60.6%)	23 (21.1%)	20 (18.4%)	109 of 133 (82.0%)
Adolescents ($n = 120$)	62 (51.7%)	45 (37.5%)	13 (10.8%)	120 of 153 (78.4%)
p-value	0.0009	0.0345	0.0025	0.1968

ISHLT Grade 2R/3R rejection is considered moderate-to-severe cellular rejection. HT, heart transplantation; RSBMSR, routine surveillance biopsy-diagnosed moderate-to-severe cellular rejection.

The results of the current study are contrary to our initial hypotheses. Instead of "symptoms" and "objective evidence of graft dysfunction" as the most common indications for EMB, "routine" EMB was the most common indication for biopsy, comprising 76% of all reported rejection episodes during the study period. When looking at only reported MSR, 81.6% of all MSR was diagnosed on "routine" EMB. In other words, <20% of moderate-to-severe ACR episodes were diagnosed in symptomatic patients or those with objective evidence of graft dysfunction. These findings may aid in the debate over the appropriate frequency of RSB after HT.

When analyzing the results by age at time of HT, infants had the greatest freedom from rejection at 4 years post-HT, followed by children and adolescents, respectively. Infants showed a steady and significantly lower hazard compared with both children and adolescents. The HRs for children and especially adolescents were highest in the first year, but declined slowly to that of infants by 3 years post-transplant (Figure 2). This result is consistent with previous studies showing the lowest overall incidence of rejection in infant recipients.[1,2,5]

Perhaps the most unexpected finding contrary to our initial hypothesis is the trend seen in infants as it pertains to the association of RSBMSR prevalence and time from HT. Previous studies have clearly shown that the first year after HT carries the highest incidence of rejection, with a significant drop-off over time.[1–3] Although this pattern holds true for all age groups, infants are thought to have the lowest prevalence of rejection at >5 years after HT compared with children and adolescents. Our results confirm the expected trend for children and adolescents. However, the trend seen in infants did not follow the expected pattern. The lowest prevalence of RSBMSR was seen in the first year, whereas the highest prevalence was seen beyond 5 years post-HT.

There is no obvious or clear explanation for this finding. In general, infant transplant recipients are thought to have a less pronounced immune response to the donor organ due to the relatively immature immune system at the time of transplant. At some institutions, this may lead to a management strategy of lower immunosuppression targets, single-drug therapy and decreased frequency of rejection surveillance in infant recipients. Higher late-term RSBMSR in infants is likely multifactorial and requires further investigation.

Another significant finding from our study is the strong association between first-year RSBMSR and risk of subsequent rejection episodes, with an impressive 21-fold increased risk of a future MSR episode. Early rejection is a well-known risk factor for subsequent episodes,[8,12–14] but never before has the impact been shown so clearly in the pediatric HT population on RSB. Only 8.7% of those surviving first-year RSBMSR were free from subsequent RSBMSR at Year 4, whereas 91% of those without RSBMSR in the first year were free from MSR at Year 4. Surviving RSBMSR in the first year places patients at a significant risk of subsequent rejection in later years,

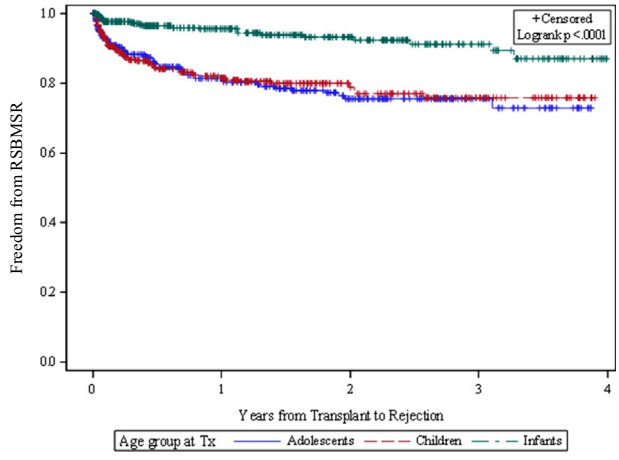

Figure 2 Freedom from routine surveillance biopsy-diagnosed moderate-to-severe cellular rejection (RSBMSR) by age and time after heart transplantation (HT).

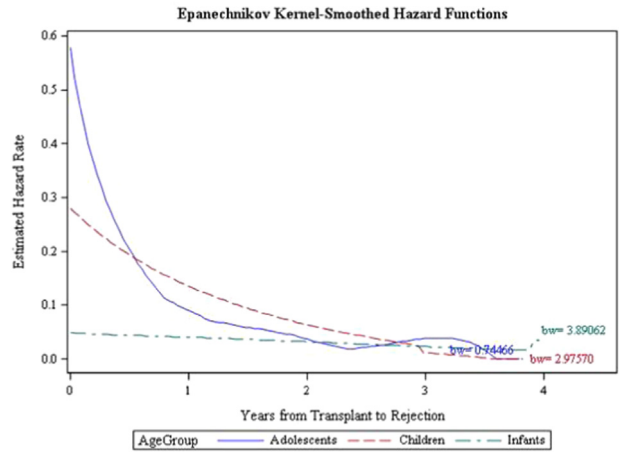

Figure 3 Hazard function of routine surveillance biopsy-diagnosed moderate-to-severe cellular rejection (RSBMSR) by age and time after heart transplantation (HT).

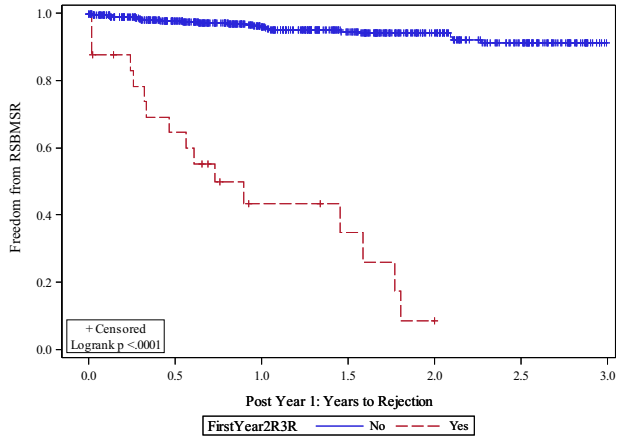

Figure 4 Freedom from subsequent routine surveillance biopsy-diagnosed moderate-to-severe cellular rejection (RSBMSR) with 1-year conditional survival of RSBMSR.

regardless of age at transplant. This finding, along with the prevalence trend just presented, seemingly negates the presumed protective effect against long-term rejection in infant recipients.[15]

Our results raise the question of how to incorporate these findings into routine rejection surveillance protocols. In light of the high risk of recurrence, patients with RSBMSR in the first year after HT should be placed on a higher intensity surveillance protocol. Many centers already increase the frequency of RSB in patients with a history of rejection. What is less clear is the necessary length of time to continue more frequent surveillance after the first rejection episode. Our follow-up analysis was limited to 4 years after HT, as the PHTS study variable was not introduced until 2010. Extended data collection and analyses are needed to assess the long-term impact of early RSBMSR on subsequent episodes of rejection. Wagner and colleagues[8] showed similar results for Years 2 to 5 after first-year rejection, but the incidence of rejection beyond Year 5 after HT was the same for those with and without early rejection. Further analysis of the PHTS database is needed to evaluate the prevalence of RSBMSR over a longer time frame and to determine the long-term risks in our patient population. Overall, our study results support a reduction in surveillance protocol intensity in pediatric heart transplant recipients, at least until Year 4, in those without RSBMSR in the first year after HT. However, infants may require more frequent RSB beyond Year 5 if longitudinal studies confirm an elevated risk of late-term RSBMSR. Additional research is needed to understand the reasons for increased risk of later RSBMSR in infants as well.

There are several strengths to our study. Data were drawn from the largest pediatric heart transplant database available, containing the majority of US transplant recipients and an ever-growing number of international recipients. This resulted in a large sample size and eliminated the potential for single-center–based conclusions that may not apply to the entire pediatric HT population. Furthermore, 86% of patients diagnosed with RSBMSR were on tacrolimus at the time of biopsy, making this the first multicenter investigation on this topic in the primarily tacrolimus-based era. Previous single-center studies have shown a decreased incidence of positive surveillance biopsies (at least during the first year) in patients treated with tacrolimus compared with cyclosporine.[8,10]

This study contained the expected limitations that accompany a retrospective database study. A significant amount of potential data were excluded due to lack of the main study variable. Indication for biopsy was not listed on 101 rejection forms, and 68 additional forms were excluded for failing to fulfill the study definition of rejection. Indication for biopsy was not included in the PHTS until 2010, making it impossible to include patients with rejection before that date. In addition, it is possible that the increased prevalence in RSBMSR seen in infants beyond Year 5 after HT may be secondary to decreased biopsy frequency. However, unpublished survey data from a companion study to this project show consistency between the first year and beyond Year 5 after HT with regard to center-specific RSB frequency. We also did not know which PHTS centers used single-drug immunosuppression, which also may have impacted the prevalence of late RSBMSR in infants. Finally, we could not control for center-specific variations in surveillance protocol intensity after a rejection episode, which may have impacted the results of our risk of subsequent RSBMSR analysis.

Conclusions

RSB detects MSR in all age groups after pediatric HT, even with long-term follow-up. Freedom from RSBMSR at 4 years after HT was higher for infant recipients compared with children and adolescents, but infants also showed the greatest prevalence of RSBMSR beyond Year 5 post-HT. Transplant recipients with RSBMSR in the first year after HT are at high risk for recurrent rejection regardless of age at HT. These findings support continuation of a higher frequency surveillance protocol beyond the first year for those patients with MSR in the first year, while also questioning the utility of frequent RSB in patients without MSR in the first year. Further study is needed to determine the impact of RSBMSR on long-term morbidity and mortality.

Disclosure statement

The authors have no conflicts of interest to disclose.

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