Accepted: 27 July 2017

DOI: 10.1111/petr.13034

ORIGINAL ARTICLE

WILEY

The impact of ischemic time on early rejection after pediatric heart transplant

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Abstract

Prolonged graft ischemia may be a risk factor for early rejection post-HTx, but this has not been well studied in children. Furthermore, factors moderating the association between IT and early rejection have not been investigated. From 2004 to 2012, pediatric HTx recipients (n = 2381) were identified from the UNOS database. A ROC curve determined the optimal IT discriminating patients by the presence of early rejection. Separate univariate analyses identified factors associated with: (i) early (prior to hospital discharge) rejection, and (ii) IT. A multivariable logistic regression assessed independent risk factors for early rejection. We included interaction terms to evaluate whether IT's independent risk effect on early rejection is moderated via interaction with associated factors found in univariate analysis. Longer IT was associated with an increased risk of early rejection. In multivariable analysis, IT > 3.1 hours was an independent risk factor for early rejection (AOR 1.44, P = .01). No interaction terms between IT and any associated factors were significant. Longer IT is an independent risk for early rejection in pediatric HTx recipients. Better understanding the association between IT and early rejection may identify interventions to mitigate this risk.

KEYWORDS

IT, pediatric heart transplant, rejection

1 | INTRODUCTION

While HTx is now standard of care for end-stage heart failure in children, the potential benefit of HTx is limited by the scarcity of donor organs. This scarcity is compounded by geographic limits imposed by graft sensitivity to prolonged ischemia. Based on early studies, an IT of <4 hours had been targeted for cardiac grafts, as longer ITs were associated with significantly higher 30-day mortality.[1,2] While several recent studies support this association of inferior outcomes with longer graft ischemia,[3-6] the mechanism by which prolonged ischemia may lead to decreased survival has not been well defined. Outcomes analyzed in these studies included primary graft failure, graft loss, and mortality; however, there is a paucity of information regarding the association between IT and rejection. The hypothesis that prolonged ischemia leads to increased risk of rejection is biologically plausible, as ischemia reperfusion injury is known to activate the innate immune system, with downstream activation of the B- and T-cell lymphocyte mediators of allograft rejection.[7]

To evaluate the impact of IT on the incidence of rejection, we retrospectively analyzed a large cohort of pediatric HTx patients to determine whether IT was associated with an increased risk of rejection. To further investigate the mechanism by which IT may increase the risk of rejection, we evaluated the interaction of several patient and donor factors with IT on the risk of rejection.

Abbreviations: AMR, antibody-mediated rejection; AOR, adjusted odds ratio; DAMP, damage-associated molecular pattern; ECMO, extracorporeal membrane oxygenation; HTx, heart transplantation; IQR, interquartile range; IT, ischemic time; PRA, panel reactive antibody; ROC, receiver operating characteristic; UNOS, United Network for Organ Sharing; VAD, ventricular assist device.

2 | METHODS

A retrospective review was performed in pediatric HTx recipients aged 0-18 years who were transplanted from 2004 to 2012 using the UNOS database. Patients with missing/unavailable IT and PRA (n = 539) were excluded, as were repeat transplants (n = 243), multiorgan transplants (n = 19), and heterotopic transplants (n = 2). Table 1 lists the donor and recipient characteristics that were collected and analyzed.

The primary end-point was rejection prior to hospital discharge, which we refer to as early rejection, and rejection at 15 months. We chose to focus on short-term rejection based on previous published data that examined IT's influence on short-term survival,[5] postulating that an effect of IT would most likely be seen in the first year. Rejection was defined as a drug-treated episode of acute rejection prior to discharge. This definition was chosen because of the variability of staining for AMR and center variability for obtaining biopsies. Therefore, a diagnosis of rejection could be made by biopsy, echocardiogram, or clinical findings. In the UNOS database, 35 patients who had unspecified graft failure recorded as either a diagnosis or cause of death, without otherwise having any rejection recorded, were excluded from the analysis with rejection in the first 15 months of transplant.

2.1 | Statistical analysis

Data are reported as frequency with percentage for categorical variables and median with IQR for continuous variables. A ROC curve determined the optimal IT discriminating patients by the presence of early rejection based on the best combination of sensitivity and specificity from the curve. Separate univariate analyses were performed to identify factors associated with early rejection and with longer IT using the optimal cutoff determined by the ROC curve, using chi-square test for categorical variables and Wilcoxon rank-sum test for continuous variables. Factors associated with early rejection ($P < .1$) in univariate analysis were included in a multivariable logistic regression to determine an independent association with early rejection. To evaluate whether there is a factor moderating the association between IT and early rejection, the multivariable logistic model specifically included interaction terms for IT and the variables with significant associations ($P < .05$) with both IT and early rejection in univariate analysis. All analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA), with statistical significance set at $P < .05$ using a two-sided test.

TABLE 1 Recipient, donor, and transplant factors analyzed

Recipient factors	Donor and transplant factors
Age at transplant	Age
Sex	Donor/recipient weight ratio
Race	Cause of death
Primary diagnosis	Infection
Status at listing	IT
Support with VAD and/or ECMO ventilator	
Inotropes pretransplant	
Pretransplant PRA	
Bilirubin	
End-stage renal disease requiring dialysis	

3 | RESULTS

3.1 | Patient characteristics

Of a total of 3184 patients transplanted during the study period, 2381 patients were included in the analysis, with exclusion of 803 patients due to the reasons described in the methods. Patient characteristics are described in Table 2. Slightly more than half of the patients (53.9%) had a primary diagnosis of cardiomyopathy. The median IT was 3.5 hours (IQR 2.8-4.3). The overall incidence of rejection was 15% prior to hospital discharge, and 30% within the first 15 months. The median length of hospital stay after HTx was 17 days (IQR 12-31 days). The median follow-up time was 11.9 months (IQR 10.6-12.6 months) with 88% remaining alive at 15 months after HTx. The population of patients that were excluded due to missing/unavailable data, as well as retransplant and multiorgan transplant, differed from the study population in terms of demographics. In addition, the excluded population had more episodes of rejection prior to discharge compared to the study cohort (18.6% vs 15.0%; $P = .0004$) and rejection within 15 months of transplant (37.9% vs 30.0%; $P < .0001$); however, they were similar in terms of death or retransplant due to rejection within 1 year of transplant (excluded cohort 2.5%, study cohort 2.1%; $P = .47$), as well as final patient status (alive, deceased, lost to follow-up, retransplantation).

3.2 | Univariate exploration of factors associated with rejection and IT

Longer IT had a significant association with increased risk of rejection prior to hospital discharge (odds ratio 1.55, $P = .001$). IT had no impact on the incidence of rejection by 15 months (odds ratio 1.09, $P = .37$). The optimal IT discriminating rejecting from non-rejecting patients by a ROC curve was 3.1 hours (area under the curve = .55). This value had a sensitivity of 74.4% and a negative predictive value of 88.5%. Additional factors associated with rejection were age at HTx ($P < .0001$), UNOS listing status at HTx ($P = .003$), underlying diagnosis ($P = .04$), VAD support at HTx ($P = .01$), PRA level ($P < .0001$), creatinine ($P = .0005$), and donor age ($P < .0001$).

Using the optimal determined cutoff for IT of 3.1 hours, the following patient, graft, and donor factors were found to be associated with a prolonged IT in univariate analysis: recipient age at HTx >2 years ($P = .02$), underlying diagnosis ($P < .0001$), dialysis ($P = .001$), PRA >10% ($P = .004$), and donor age ($P = .01$). The analysis was replicated using the cohort's median IT of 3.5 hours with no notable differences in results.

TABLE 2 Patient and clinical characteristics (N = 2381)

Age at transplant, y	
0-1	918 (38.6)
2-18	1463 (61.4)
Male sex	1307 (54.9)
Race	
Caucasian	1314 (55.2)
African American	535 (22.5)
Hispanic	382 (16.0)
Asian	82 (3.4)
Status at listing	
1 or 1A	1552 (65.2)
1B	316 (13.3)
2	483 (20.3)
Primary diagnosis at transplant	
Cardiomyopathy	1283 (53.9)
Congenital heart defect	1009 (42.4)
Others	89 (3.7)
Waiting time, d	44 (16-102)
Support at transplant	
VAD	387 (16.3)
ECMO	147 (6.2)
Inotropes	1151 (48.3)
Ventilator	388 (16.3)
Dialysis at transplant	87 (3.7)
Creatinine at transplant	0.5 (0.3-0.7)
Total bilirubin at transplant	0.7 (0.4-1.2)
Maximum PRA prior to transplant, %	
0	1578 (66.3)
1-10	306 (12.9)
11-49	268 (11.3)
>50	229 (9.6)
IT, h	3.5 (2.8-4.3)
Donor characteristics	
Donor age, y	
0-1	823 (34.6)
2-18	1558 (65.4)
Donor/recipient weight ratio	1.26 (1.03-1.58)
Outcome measures	
Acute rejection episode(s) prior to hospital discharge	
No	2021 (84.9)
Yes	356 (15.0)
Unknown	4 (0.2)
Any evidence of rejection episode(s)[b] within 15 mo of transplant	715 (30.0)
Death or retransplant due to rejection within 1 y of transplant	49 (2.1)
Total deaths within 1 y of transplant	248 (10.4)
Time from transplant to discharge, d	17 (12-31)
Time from transplant to last follow-up within 15 mo of transplant, mo	11.9 (10.6-12.6)
Final patient status within 15 mo of transplant	
Alive	2096 (88.0)
Deceased	249 (10.5)
Lost to follow-up	16 (0.7)
Retransplant	20 (0.8)

[a]Data are presented as N (%) for categorical variables and median (IQR) for continuous variables

[b]Including deaths and retransplants due to rejection.

3.3 | Multivariable analysis

In addition to all characteristics possessing a significant univariate association with rejection, the initial multivariable logistic regression model included interaction terms for IT and the variables associated with both increased IT and early rejection: recipient age, donor age, PRA, and cardiac diagnosis. Because none of these interaction terms moderated the effect of IT on the risk of rejection (Table 3), the final multivariable model included only the discrete variables associated with rejection on univariate analysis. In the final multivariable model, factors independently associated with an increased frequency of acute rejection prior to hospital discharge were IT >3.1 hours, recipient age >2 years, and PRA >10% (Table 4).

4 | DISCUSSION

The key finding of this study was that longer IT is an independent risk factor for rejection prior to hospital discharge in pediatric HTx recipients. The risk conferred by IT was not moderated by any other factors in our analysis. IT, for the purposes of our study, was analyzed as a

TABLE 3 Interaction of patient factors and IT on risk of rejection

Characteristics	AOR	*P*-value
Recipient age × IT		
2-18 vs 0-1 with IT >3.1 h	1.72	.67
2-18 vs 0-1 with IT <3.1 h	2.07	
Primary diagnosis × IT		
CHD vs CM with IT >3.1 h	1.26	.95
CHD vs CM with IT <3.1 h	1.28	
PRA × IT		
PRA >10% vs ≤10% with IT >3.1 h	2.27	.09
PRA >10% vs ≤10% with IT <3.1 h	1.31	
Donor age × IT		
Donor age <1 vs 1-18 with IT >3.1 h	1.49	.26
Donor age <1 vs 1-18 with IT <3.1 h	.83	

P-value from multivariable logistic regression.

TABLE 4 Independent risk factors for rejection

Characteristics	AOR	CI	*P*-value
IT >3.1 h	1.44	1.10-1.88	.01
Recipient age 2-18	1.81	1.24-2.62	.002
PRA≥10%	1.99	1.53-2.60	<.0001

P-value from multivariable logistic regression.

dichotomous variable. While there was no clear time point to discriminate rejecting and non-rejecting, an IT of 3.1 hours was the single best time point. Nonetheless, our conclusion that a longer IT is associated with a higher risk of rejection is valid.

Several studies have examined the impact of IT on survival with contradictory results. A single-center study from Loma Linda University compared the outcome of 14 pediatric HTx recipients with donor IT >8 hours to 14 with short IT (<90 minutes) and found no association between prolonged IT and outcome at 5 years post-HTx.[8] Another study from Columbia University found no association between IT, transplant coronary disease, or survival in adults.[9] These single-center studies contrast with other studies which have found inferior outcomes in heart recipients of grafts with longer IT, most notably a large pediatric study (n = 4716 patients) by Ford et al,[5] which showed that IT >3.5 hours were associated with a 30% higher graft loss within 6 months of transplant.

Given that an association between IT and survival post-HTx appears possible, the question of whether IT is associated with rejection merits consideration. To our knowledge, this specific association has not been previously examined in depth in a large pediatric dataset. Our finding that increased IT (>3.1 hours) is independently associated with rejection prior to hospital discharge is novel. Of the aforementioned studies regarding IT and overall outcomes, only one study[3] compared the incidence of rejection between long and short IT groups. These investigators found no difference in rejection episodes in the first year after transplant between groups; however, rejection prior to discharge was not specifically evaluated in that study. Another study with 245 patients post-HTx found no difference in rejection (either AMR and CR) in patients with a prolonged IT (mean IT 268 minutes) vs short IT; however, graft survival was significantly worse in the prolonged IT group.[10] Rejection incidence was assessed at mean follow-up of 9 years, with rejection prior to discharge not specifically evaluated. In the present study, we found no association between rejection and IT at 15 months post-HTx. This could be due to the acute risk related to ischemia reperfusion injury mediated inflammation and immune activation,[7] being eventually outweighed by other risk factors as a patient moves beyond the immediate post-HTx period.

Ischemia reperfusion injury is a possible mechanism by which IT may be associated with early rejection. Ischemia reperfusion injury activates the innate immune system, which in turn activates the adaptive immune response via a sequence of events that begins with the release of DAMPs. This stimulates pattern recognition receptors (including Toll-like receptors),[7,11,12] which results in the maturation of antigen-presenting cells and ultimately results in the activation and differentiation of T cells into effector T-helper cells, which are involved in acute rejection. Furthermore, dying donor dendritic cells may be a source of alloantigens for recipient antigen-presenting cells that stimulate T cells.[11]

To understand the mechanism by which IT may increase the risk for rejection, we performed an investigation into the interaction of different patient and donor characteristics with IT. We postulated that elevated PRA would reflect a state of immune activation that would be amplified by the inflammatory response caused by ischemia reperfusion injury. We specifically hypothesized that IT and PRA would interact to enhance the risk of rejection, beyond the additive risks that each conveys individually. While IT and PRA trended toward an interaction ($P = .09$), this was not statistically significant.

4.1 | Implications

While this study did find an independent association between IT and early rejection, given the significant donor shortage in pediatric HTx and high wait-list mortality for those awaiting HTx, we would not advocate using the results of this study to decline otherwise reasonable organ offers strictly based on longer ITs. However, an individual receiving a graft with a prolonged IT may benefit from increased early rejection surveillance or increased immunosuppression post-HTx. Ultimately, alternative preservation strategies such as ex vivo perfusion may prove to be the best solution to the problems associated with cold ischemia. This technology, which is currently being developed, has been shown to be clinically feasible and as safe as cold storage in a prospective randomized trial.[13] However, there is currently no evidence to suggest that ex vivo perfusion is superior to cold ischemia for rejection or other outcomes.

4.2 | Limitations

This study has the typical limitations inherent to a retrospective database study. We were limited to the information collected in the database. Incomplete data is one of these limitations, which often is not a random occurrence. The population of patients that were excluded due to missing/unavailable data, as well as retransplant and multiorgan transplant, differed from the study population as described in the methods and results. Secondly, we could only determine the incidence of rejection in general and not of cellular or AMR, which could have provided valuable insight. Information regarding induction therapy was not available in the UNOS database. Cross-match results would be of interest, but this is not a standard data field in the UNOS database and, therefore, could not be included in our analysis. Because of individual center variability in staining biopsy specimens for the presence of AMR, we decided to define rejection clinically based on treatment for rejection rather than biopsy score. This may result in an inexact estimate of the incidence of rejection. Lastly, one of the primary outcomes of the study, acute rejection prior to hospital discharge after transplant, may be influenced by the length of hospitalization after transplant. It is possible that patients who had a prolonged hospitalization due to complications other than rejection may have had more biopsies and therefore a higher incidence of rejection prior to hospital discharge.

5 | CONCLUSION

Longer IT is associated with an increased risk of rejection prior to hospital discharge in pediatric patients. The mechanism by which IT increases the risk of rejection needs further characterization.

AUTHORS' CONTRIBUTIONS

H. Sonali Magdo: Concept/design, performed data analysis and interpretation, drafted the article, and approved the submitted and final version of the article; Joshua M. Friedland-Little: Concept/design, performed data analysis and interpretation, performed critical revision of article, and approved the submitted and final version of the article; Sunkyung Yu: Study design and statistics, performed data analysis and critical revision of article, and approved the submitted and final version of the article; Robert J. Gajarski: Study concept, performed critical revision of the article, and approved the submitted and final version of the article; Kurt R. Schumacher: Concept/design, performed data interpretation and critical revision of the article, and approved the submitted and final version of the article.

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How to cite this article: Magdo HS, Friedland-Little JM, Yu S, Gajarski RJ, Schumacher KR. The impact of ischemic time on early rejection after pediatric heart transplant. *Pediatr Transplant*. 2017;21:e13034. https://doi.org/10.1111/petr.13034