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Gene Expression Monitoring in Pediatric Heart Transplant Recipients

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Abstract

Background: AlloMap® gene expression testing is a non-invasive screening tool approved for use in heart transplant recipients age 15 and older. Experience with AlloMap® in pediatric heart transplant recipients is limited. We sought to describe the variations in AlloMap® scores seen in pediatric heart transplant recipients.

Methods and findings: This is a retrospective study of all pediatric heart transplant recipients with AlloMap® scoring at a single institution between 2013 and 2014. All possible scores were recorded. Other variables recorded at the time of each AlloMap® score included immunosuppressive regimen, patient demographics and endomyocardial biopsy (EMB) results. Patients were excluded if they had undergone other solid or multi-solid organ transplantation. One-hundred AlloMap® scores were available from 42 patients, with a median age at transplantation of 4.3 years. The median AlloMap® score for all patients was 32 (IQR, 30-35). Of the 100 AlloMap® scores, 10% were collected in patients <2 years, 41% in 2-12 years and 49% were >12 years of age. There was little difference in the median score between age groups (p=0.143). Forty-five scores had a concomitant biopsy. Twenty-eight (62%) patients had ISHLT grade 0 and 16 (36%) had ISHLT grade 1 rejection. AlloMap® scores were higher in patients with evidence of ISHLT grade 1 acute cellular rejection (ACR) on EMB (p=0.044). AlloMap® scores were similar across all immunosuppression regimens (p=0.403), with TAC+MMF (n=43) and TAC+SIR (n=27) being the most commonly used regimens. In patients with multiple AlloMap® readings, the median change in AlloMap® score from baseline reading was 2 (IQR, 2-5) without significant change on biopsy findings.

Conclusions: In pediatric heart transplant recipients, AlloMap® scores were higher in patients with ISHLT grade 1 rejection than in patients with ISHLT grade 0 rejection. AlloMap® scores did not appear to be affected by patient age or immunosuppression regimen. Further studies should be performed to confirm the findings of this study and determine the place for AlloMap® in post-transplant monitoring of pediatric patients.

Keywords: AlloMap®; Pediatric heart transplant; Gene expression; Immunosuppressive

Abbreviations: ACR: Acute Cellular Rejection; AMR: Antibody Mediated Rejection; AZA: Azathioprine; EMB: Endomyocardial biopsy; ISHLT: International Society for Heart and Lung Transplantation; MMF: Mycophenolate mofetil; MUSC- Medical University of South Carolina; PRED: Prednisone; TAC: Tacrolimus; SIR: Sirolimus

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Introduction

Rejection is a leading cause of morbidity in pediatric heart transplant recipients, often through progression to graft failure and death. In the most recent era, 15% of patients experienced treated rejection within the first year post-transplant, with

rates of rejection being highest in patients greater than 1 year of age [1]. Frequent monitoring with cardiac catheterization and endomyocardial biopsy (EMB) has been utilized to monitor patients in the post-transplant period for evidence of rejection [2]. However, EMB has been associated with risks such as tricuspid valve damage and regurgitation, conduction system abnormalities, and cardiac perforation [3-5]. Endomyocardial biopsy is subject to sampling error and inter-observer variability, and can only detect acute cellular rejection (ACR) once cellular infiltration and damage has occurred [6,7]. Major complications have also been reported with cardiac catheterization including arrhythmias, hemodynamic compromise, perforation, and death [8,9]. During these procedures, patients are exposed to high doses of ionizing radiation which may increase the child's risk of cancer development during their lifetime [10]. Additionally, patients require hospitalization for monitoring after this invasive procedure, incurring an average hospital cost of $1200-$5600 per biopsy [6].

AlloMap® Molecular Expression Testing (CareDx, Brisbane, CA) is an innovative, non-invasive method for determining the risk of rejection in adult and adolescent heart transplant recipients ages 15 years and older and at least 2 months post-transplant [7,11-13]. However, experience in pediatric heart transplant recipients is limited.

The primary aim of this project was to evaluate AlloMap® scores and determine the correlation of these scores with EMB results in pediatric heart transplant patients. The secondary aims of this project are to determine what transplant-related factors, if any, alter the reliability of AlloMap® scores in the pediatric heart transplant population and to describe the use of various immunosuppressive regimens at this institution and the relationship of these regimens and their effects on the patient's AlloMap® score.

Methods

Patients

Pediatric patients (age<20 years) with a prior heart transplant and monitored by AlloMap® scoring and EMB at Medical University of South Carolina (MUSC) between April 1, 2013 and December 31, 2014 were considered for inclusion in this study. All possible AlloMap® scores were recorded. Patients were excluded if they had undergone other solid or multi-solid organ transplantation. The MUSC Pediatric Heart Transplant protocol includes AlloMap® scoring performed in conjunction with EMB at months six and nine post-transplant. Once patients reach one year post-transplant, AlloMap® is performed twice yearly, not in conjunctlion with a biopsy. More frequent AlloMap® scoring is performed with a recent history of ACR. For study purposes, rejection was defined as any evidence of ACR on EMB (ISHLT Grade ≥ 1A). Biopsies reviewed during the study time period were graded based on the 1990 ISHLT Standardized Cardiac Biopsy grading. Patient data were obtained through retrospective review of the electronic medical record. Data included patient demographics, indication for and date of transplant, immunosuppressive regimen and corresponding serum trough concentrations, viral load, and allograft monitoring studies. Assessment of the corresponding serum trough concentration was patient-specific, with target concentrations based upon time post-transplant and the patient's history of rejection, with higher trough concentrations desired in the first six months to one year post transplant. AlloMap® testing results were obtained from the AlloMap® database. The study protocol conformed to the Declaration of Helsinki and was approved by the MUSC Institutional Review Board.

AlloMap® Gene Expression Testing

The AlloMap® test utilizes a 20-gene algorithm to assess the gene expression profile of ribonucleic acid isolated from peripheral blood mononuclear cells and to aid in the identification of patients at low risk of ACR [7-13]. Eleven genes have been evaluated and shown to be associated with the presence of ACR in adult and adolescent heart transplant patients. The expression of these identified genes within the sample is then converted to a score ranging from 0-40, with thresholds for identifying rejection determined by the individual transplant center based on experience with AlloMap® and the negative predictive value desires. No pre-specified threshold existed at this institution given the investigational nature of this testing in pediatric patients. All samples were collected and sent to CareDx Laboratory in Brisbane, CA for analysis.

Statistical analysis

Patient demographics, AlloMap® scores, and EMB results were analyzed descriptively and expressed as frequencies or medians with interquartile ranges. Kruskal-Wallis Tests were used to determine the relationship of AlloMap® scores to endomyocardial biopsy result, age of patient at time of initial AlloMap® score, and immunosuppressive regimen. A p-value less than 0.05 was considered significant.

Results

Forty-two pediatric heart transplant recipients met inclusion criteria, with 21 male and 21 female patients included in the analysis **(Table 1)**. One hundred AlloMap® scores were collected

Table 1 Patient Demographics.

Number of Patients	42
Number of AlloMap® samples	100
Race	
White	24 (57.1%)
Black	16 (38.1%)
Hispanic	1 (2.4%)
Asian	1 (2.4%)
Gender	
Male	21 (50%)
Female	21 (50%)
Median Age at Transplant	4.3 years (0.7-9.9 years)
Time since transplant at AlloMap® collection	2.7 years (0.3-14.3 years)
Age at AlloMap® collection	4 (10%)<2 years
	17 (41%) 2-12 years
	21 (49%)>12 years

amongst the 42 pediatric patients with a median age at transplant of 4.3 years and median time from transplantation to AlloMap® score of 2.7 years (range 0.3-14.3 years). The median AlloMap® score for all patients was 32 (IQR, 30-35). Ten percent were collected in patients <2 years, 41% in 2-12 years and 49% were >12 years of age. There was little difference in the median score between age groups [<2 years: 34(IQR, 33-36), 2-12 years: 33 (IQR, 30-35), >12 years: 32 (IQR, 29-35), p=0.143].

Forty-five scores had a concomitant biopsy; twenty-eight (62%) patients had ISHLT grade 0 and 16 (36%) had ISHLT Grade 1 rejection (9 patients with Grade 1A and 7 patients with Grade 1B). AlloMap® scores were significantly higher in patients with evidence ISHLT Grade 1 rejection vs. ISHLT Grade 0 rejection on EMB **(Table 2)**. Only one patient with ISHLT grade 1B rejection on biopsy received treatment for ACR at the time of AlloMap® scoring. This patient had an AlloMap® score of 38, with new donor-specific antibodies and an elevated B-type natriuretic peptide. One patient with ISHLT grade 1A and one patient with ISHLT grade 1B rejection, both with AlloMap® scores of 35, did progress to clinical rejection at 4 months and 12 months post-biopsy and AlloMap® scoring. There was a single episode of ISHLT Grade 2 rejection, which coincided with an AlloMap® score of 36. This patient received bortezemib and rituximab with plasmapheresis for antibody mediated rejection (AMR) two months prior to AlloMap® scoring, with successive monthly intravenous immune globulin. Intravenous immune globulin was the only therapy received after this AlloMap® score was obtained with no recurrence of AMR or development of ACR that required medical intervention.

Immunosuppression regimen was recorded at the time of each AlloMap®. Tacrolimus with mycophenolate mofetil (TAC+MMF) and tacrolimus with sirolimus (TAC+SIR) were the most commonly used immunosuppressive regimens **(Table 3)**. Other regimens utilized included prednisone with tacrolimus and mycophenolate (PRED+TAC+MMF) and tacrolimus with azathioprine (TAC+AZA). Serum trough concentrations were reviewed if collected at the time of AlloMap® screening and assessed against the patient's specific goals of therapy. At the time of AlloMap® screening, tacrolimus trough concentrations were considered therapeutic in 43% of samples, while sirolimus trough concentrations were considered therapeutic in 43% of samples. AlloMap® scores were similar across all immunosuppression regimens (p=0.403).

Twenty-eight of the 42 patients included for analysis had multiple AlloMap® scores throughout the study period. The median change in AlloMap® score from baseline was 2 points (IQR 2-5), which correlates to a 6.4% intrapatient variability (range 0-52%). Despite this variability, only one episode of ISHLT Grade 2 rejection occurred.

Table 2 AlloMap® Score by Rejection Severity.

	Median AlloMap® Score (IQR)	*p*- value
ISHLT Grade 0, n=28	31.5 (29-35)	0.044
ISHLT Grade 1, n=16	35 (32-36)	
ISHLT Grade 2, n=1	36	

Table 3 AlloMap® Score by Immunosuppressive Regimen.

Immunosuppression Regimen	Median Time Post-Transplant	Median AlloMap® Score (IQR)	*p*-value
Prednisone + TAC + MMF, n=15	1.47 years	31 (27-35)	0.403
TAC + MMF, n=43	1.52 years	33 (30-35)	
TAC + SIR, n=27	6.65 years	31 (29-34)	
TAC + AZA, n=9	3.88 years	34 (32-37)	
Other regimen, n=6	5.38 years	34 (28-36)	

Discussion

To our knowledge, this is the first report of the use of AlloMap® testing as an adjunct to traditional monitoring in patients less than 15 years of age. In this retrospective study of 42 pediatric heart transplant patients aged 1-19 years and at least 6 months post-transplant, we found that activity on the biopsy correlated with higher AlloMap® scores, as patients with Grade 1 rejection had higher scores than those with Grade 0 rejection on EMB. Our study primarily compared Grade 0 and Grade 1 rejection found on biopsy. This differs from the previously conducted adult studies, which defined rejection as ISHLT grade 3 or higher and tested the ability of AlloMap® to distinguish the presence of moderate-to-severe rejection (ISHLT Grade ≥ 3) from no rejection (ISHLT grade 0) [7]. However, when all biopsy scores were analyzed in the CARGO study, it was found that ISHLT Grade 1B correlating AlloMap® scores were higher than those of ISHLT Grades 0, 1A and 2, but similar to those associated with ISHLT Grade 3A [7]. A similar correlation was seen in our population with evidence of ISHLT Grade 1 rejection having higher corresponding AlloMap® scores than ISHLT grade 0. While this correlation was statistically significant, the study authors note that this may not be of great clinical significance, as the patients included in this study would have biopsy grading classified as grade 0R or grade 1R (no rejection or mild rejection) based on the 2004 revision of ISHLT ACR grading. Although this grade of rejection may not require treatment in all cases, three patients (one with grade 1A and two with grade 1B rejection) in this study required treatment for ACR within 12 months of EMB and AlloMap® screening [14]. AlloMap® has not been validated for the detection of mild rejection, but rather to distinguish the presence of moderate-to-severe rejection [7]. The relationship of AlloMap® scoring across all grades of rejection in our pediatric population is unclear as there were no episodes of ISHLT Grade 3 rejection during the study period.

The AlloMap® test utilizes a mathematical equation to develop an algorithm that is used to provide a value between 0 and 40 scoring range [13]. The AlloMap® scores collected in our population ranged from 14 to 38. When comparing these scores to the AlloMap® Testing Clinical Performance Characteristics table in patients more than 6 months post-transplant, the score ranges suggest that there is an estimated probability of 98.2% to 100% that patients are not experiencing ISHLT grade 3 rejections [13]. This was true of our population, as we had no incidence of ISHLT grade 3 rejections.

In the IMAGE trial, only 17.6% of rejection episodes were detected by AlloMap®, leading the authors of that study to suggest that clinical diagnosis, rather than change in the AlloMap® score, may detect the majority of serious rejection episodes [15]. The previously conducted adult trials did not examine the intrapatient variability of AlloMap® scores to be

expected within samples without significant changes on EMB or in allograft function. Twenty-eight patients included in this analysis had multiple AlloMap® scores performed which found wide intrapatient variability in the reported AlloMap® score without change on EMB. This insignificant variability in scoring in addition to the previously reported data by the IMAGE trials questions the reliability of AlloMap® scoring in detecting acute cellular rejection.

Various immunosuppressive regimens were utilized in our patient population with little effect on the AlloMap® score between the groups. The most commonly utilized immunosuppressive regimens are congruent with the most recent reports of pediatric heart transplant patients internationally, with a dual-therapy regimen of tacrolimus with mycophenolate mofetil being most common [16]. Previous adult studies have shown falsely lower AlloMap® scores with the use of prednisone in doses above 20 mg daily through decreased expression of IL1R2, ITGAM, and FLT3 genes [7,13]. Nineteen patients received prednisone as part of their immunosuppressive regimen. Weight-based dosing was utilized, with patients in this cohort receiving doses of 0.05 -0.17 mg/kg/day, up to 6 mg daily at the time of blood collection. The administration of these doses of corticosteroid did not appear to alter the AlloMap® score.

Limitations

This study is the first report of use of AlloMap® scoring in pediatric patients. It was performed at a single pediatric academic institution where only 42 patients were available for analysis. Collaboration with other pediatric heart transplant groups using AlloMap® scoring would provide more robust data regarding its use in the pediatric population. Our study group had a low rate of clinically significant ACR and no occurrences of moderate-to-severe rejection, so we were unable to assess the utility of AlloMap® scoring across all grades of rejection or the ability of AlloMap® to detect the presence of moderate-to-severe rejection. Finally, AMR remains an important factor in graft survival post-transplant. AlloMap® testing does not have the ability to detect AMR, so a move to AlloMap® as a replacement for EMB in post-transplant rejection monitoring could lead to undetected and untreated AMR.

Conclusion

Our study suggests that AlloMap® gene expression testing may be useful to detect the presence of ISHLT Grade 1 rejection in pediatric heart transplant recipients who are at least 6 months post-transplant. Additional experience with AlloMap® scoring in pediatric patients is needed to confirm the results of this study characterize the role of AlloMap® in pediatric patients, validate its use across all grades of rejection, and to determine its potential for replacement of endomyocardial biopsy in post-transplant rejection monitoring.

Authors' contributions

Courtney Sutton: participated in concept/design, data collection, data analysis/interpretation, drafting the article and approval of article; Ryan Butts: participated in concept/design, data analysis/interpretation, critical revision of article, approval of article, and statistics; Ali Burnette: participated in concept/design, data analysis/interpretation, critical revision of article, approval of article; Andrew Savage: participated in concept/design, data analysis/interpretation, critical revision of article, approval of article; Walter Uber: participated in data analysis/interpretation, critical revision of article, approval of article; A. Lauren Haney: participated in data concept/design, data analysis/interpretation, drafting of article, critical revision of article, and approval of article.

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