



ORIGINAL CLINICAL SCIENCE

Eosinophil count, allergies, and rejection in pediatric heart transplant recipients

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BACKGROUND: Allograft rejection and long-term immunosuppression remain significant challenges in pediatric heart transplantation. Pediatric recipients are known to have fewer rejection episodes and to develop more allergic conditions than adults. A T-helper 2 cell dominant phenotype, manifested clinically by allergies and an elevated eosinophil count, may be associated with immunologic quiescence in transplant recipients. This study assessed whether the longitudinal eosinophil count and an allergic phenotype were associated with freedom from rejection.

METHODS: This single-center, longitudinal, observational study included 86 heart transplant patients monitored from 1994 to 2011. Post-transplant biannual complete blood counts, allergic conditions, and clinical characteristics related to rejection risk were examined.

RESULTS: At least 1 episode of acute cellular rejection (ACR) occurred in 38 patients (44%), antibody-mediated rejection (AMR) occurred in 11 (13%), and 49 patients (57%) were diagnosed with an allergic condition. Patients with ACR or AMR had a lower eosinophil count compared with non-rejectors ($p = 0.011$ and $p = 0.022$, respectively). In the multivariable regression analysis, the presence of panel reactive antibodies to human leukocyte antigen I ($p = 0.014$) and the median eosinophil count ($p = 0.011$) were the only independent covariates associated with AMR. Eosinophil count ($p = 0.010$) and female sex ($p = 0.009$) were independent risk factors for ACR. Allergic conditions or young age at transplant were not protective from rejection.

CONCLUSIONS: This study demonstrates a novel association between a high eosinophil count and freedom from rejection. Identifying a biomarker for low rejection risk may allow a reduction in immunosuppression. Further investigation into the role of the T-helper 2 cell phenotype and eosinophils in rejection quiescence is warranted.

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Allograft rejection and the long-term effects of immunosuppression remain significant challenges in heart transplantation.¹ In particular, antibody-mediated rejection (AMR) is known to cause graft dysfunction, allograft vasculopathy, and is associated with poor survival.²⁻⁴ CD4

T-helper (Th) cells and the adaptive immune response are key elements involved in cardiac allograft rejection. Among CD4 cells, 2 phenotypes exist that are defined by their cytokine profile and principal immune responses. Th1 cellular responses are largely pro-inflammatory and mediated by cytokines such as interferon- γ . Their primary role is in eliminating intracellular microbes, a process closely resembling the immune response in solid-organ rejection.⁵ Th2 cellular responses are predominately mediated by interleukin-4, -5, and -10 and are involved in allergic

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responses and fighting parasitic infections.^{6,7} In addition, T-regulatory cells (Tregs), such as CD4⁺CD25⁺ forkhead box P3⁺ cells, may be associated with tolerance and are known to interact with Th1/Th2 cells.^{8,9} Specifically, studies have shown that Th2-associated cytokines promote Treg proliferation and suppress rejection.^{10,11} The balance between Th1 and Th2 phenotypes may be important in mediating allograft rejection vs acceptance.^{9,12}

Allergic conditions are more prevalent in pediatric transplant recipients,^{13–17} and the peripheral eosinophil count is a marker of allergic conditions. Given the possible role of Th1 vs Th2 in rejection vs allergic responses, we sought to identify potential clinical markers of an underlying Th2 dominance and immunologic quiescence by examining the association between the longitudinal eosinophil count and the presence of allergic conditions with rejection history in a pediatric heart transplant cohort.

Methods

The local Institutional Review Board approved this study

Patients

This was a single-center, retrospective study of pediatric heart transplant recipients monitored from 1994 to 2011. Inclusion criteria were age at transplant <21 years, survival to 12 months post-transplant, and availability of at least 2 complete blood counts (CBCs) with differential at least 6 months apart. One set of data closest to each 6-month interval was collected from the time of transplant until the patient died, underwent retransplantation, transferred to another center, or to the end of the study period.

Laboratory studies

Patients had routine laboratory studies according to the clinical protocol that included CBCs and immunosuppression drug levels approximately once per month. Other laboratory studies described were collected every 3 to 6 months. For the purpose of the study, clinically significant neutropenia was defined by the need for filgrastim and anemia by the need for packed red blood cell transfusion or recombinant erythropoietin. Panel-reactive antibody (PRA) and donor-specific antibody (DSA) were considered positive when the mean fluorescence intensity was > 5,000 units based on internal calibration by our immunogenetics laboratory.

Immunosuppression and rejection

Of the patients included, 50% received anti-thymocyte globulin (ATG) in the first 10 days after transplant. Calcineurin inhibitors were used for primary maintenance immunosuppression. Adjunctive agents included sirolimus, mycophenolic acid, or azathioprine. Corticosteroid was discontinued within the first 3 months post-transplant. For patients who switched calcineurin inhibitors, the medication that was used longer defined the dominant immunosuppressive drug. Rejection was defined as requiring acute intensification of immunosuppressive therapy based on the clinical condition with supportive evidence from a biopsy specimen classified as acute cellular rejection (ACR) with histopathology grade $\geq 2R$ or antibody-mediated rejection (AMR) with pAMR

grade ≥ 1 by International Society for Heart and Lung Transplantation criteria.¹⁸

Allergic conditions

Allergic diseases included eczema, food allergies, asthma, eosinophilic gastroenteritis, and any other immunoglobulin E-mediated responses requiring treatment and/or avoidance of the offending agent. Eczema was included if a patient required treatment with a topical or systemic corticosteroid on a chronic or recurrent basis. Food allergies were included after a positive radioallergosorbent test and/or strict avoidance of the food substance. Asthma was included if a patient required chronic or recurrent asthma treatment. The diagnosis of eosinophilic gastroenteritis was based on endoscopy and tissue biopsy specimen.

Other conditions affecting the CBC

For patients who underwent treatment for post-transplant lymphoproliferative disorder, CBC values were omitted for 1 year after the diagnosis due to the pronounced treatment effect on the bone marrow. For patients who were treated for AMR, which typically included the use of ATG, a sub-analysis was performed on all CBCs obtained before vs after AMR treatment because many patients had early rejection, which did not allow enough CBCs to be assessed before treatment if the every 6-month data collection frequency was used. These patients' 6-month CBCs, before and after treatment, were still included in the overall analysis when available. One patient was diagnosed and treated for AMR on Post-operative Day 1 and was not included in the AMR CBC sub-analysis.

Statistics

Data are presented as mean with standard deviation if normally distributed, and otherwise, as median with the interquartile range (IQR). Categorical data are presented as frequencies with percentages. We separated the cohort by age at 3 years because this cutoff produced the most noticeable differences in CBC values. Statistical differences between groups were assessed using the Student's *t*-test for continuous normally distributed data, the Mann-Whitney *U* test for non-normally distributed data, and Fisher's exact test for categorical variables. Backward step-wise multivariable regression analyses to assess independent risk factors for rejection were performed. Age at transplant was the only term forced into the model; other covariates were entered at a *p*-value of < 0.2. A natural logarithmic transformation of the median eosinophil count was performed for the multivariable analyses. All analyses were performed using SPSS 19.0 software (IBM, Armonk, NY). A *p*-value of < 0.05 was considered statistically significant.

Results

Patient characteristics

The study criteria were fulfilled by 86 of the 127 patients who received transplants during the study period. The clinical characteristics of the study population are reported in Table 1. The median age at transplant was 14.5 months (IQR, 5.8–113.3 months). The mean duration of follow-up was 40.5 $\pm$ 21.9 months, with 6.8 $\pm$ 3.7 CBCs collected per

Table 1 Demographics and Clinical Characteristics of the Population Studied

Variables	Value (N = 86)
Sex, No. (%)	
Male	44 (51)
Female	42 (49)
Cardiovascular diagnosis, No. (%)	
Congenital heart disease	51 (59)
Cardiomyopathy	35 (41)
Age at transplant, months	
Mean $\pm$ SD	57.8 $\pm$ 67.4
Median (IQR)	14.5 (5.8–113.3)
Number of samples	
Total group	6.8 $\pm$ 3.7
Age at transplant	
< 3 years old	6.6 $\pm$ 3.6
> 3 years old	6.9 $\pm$ 3.5
Follow-up duration, months	
Total group	40.5 $\pm$ 21.9
Age at transplant	
$\leq$ 3 years old	39.8 $\pm$ 3.6
> 3 years old	41.7 $\pm$ 3.6
Dominant immunosuppressant, No. (%)	
Cyclosporin	32 (37)
Tacrolimus	54 (63)
PTLD, No. (%)	8 (9)
Treated for	
Neutropenia, No. (%)	9 (10)
Anemia, No. (%)	10 (12)
Allergic condition, No. (%)	49 (56)

IQR, interquartile range; PTLD, post-transplant lymphoproliferative disorder; SD, standard deviation.

patient. The median time from transplant to sample collection was 30 months (IQR, 12–66 months), and the median age at time of sample collection was 82 months (IQR, 34–160 months). Approximately 43 of the patients received ATG for induction and 9 patients required ATG for rejection.

Hematologic profile

Overall, an abnormally low hemoglobin, hematocrit, white blood cell (WBC), platelet, and lymphocyte count was observed compared with healthy children (Table 2).¹⁹ Compared with those who received transplants before 3 years of age, older recipients had lower WBC, platelet, lymphocyte, monocyte, eosinophil, and basophil counts. The younger age group had a lower hematocrit/hemoglobin. The median eosinophil count was widely distributed, with skewing to the higher values (Figure 1). Nineteen patients (22%) required therapy for neutropenia or anemia: 9 patients (10%) required filgrastim, and 10 (12%) required intervention for anemia. No association was found between the primary calcineurin inhibitor used and treatment for neutropenia or anemia ($p = 0.685$), and no association was found between age of patient at transplant and the need

for filgrastim. A nearly significant association was found between patients who received allografts before the age of 3 years and treatment for anemia ($p = 0.081$). Eight patients (42%) required treatment for both, suggesting these components of bone marrow suppression tend to coexist.

Allergy and eosinophil count

Allergic conditions were common in this cohort, with 49 patients (56%) having at least 1 diagnosis, comprising eczema in 33 (38%), new food allergy in 20 (23%), asthma in 16 (19%), eosinophilic gastroenteritis in 4 (5%), and other immunoglobulin E-mediated allergies in 2 (2%). An age difference in the prevalence of allergic conditions was also noted. Among those diagnosed with an allergy, 38 of 49 (78%) were younger than 3 years old when they underwent transplantation, whereas 27 of 37 (62%) patients without an allergy were older than age 3 ($p < 0.01$). With regard to the CBC components, the presence of an allergy correlated only with a higher eosinophil count.

Risk factors for rejection

During the follow-up period, 38 patients (44%) experienced ACR and 11 patients (13%) experienced AMR. Ten patients (26%) had >1 episode of ACR compared with only 1 patient (9%) with >1 episode of AMR. More than 50% of first rejection episodes occurred within the first 12 months after transplant (61% of first ACRs and 54% of first AMRs).

In the univariate analysis of risk factors associated with ACR (Table 3), only a lower eosinophil count reached statistical significance ($p = 0.011$). Variables associated with AMR included positive PRA for human leukocyte antigen (HLA)-I ($p = 0.006$) or HLA-II ($p = 0.019$), presence of DSA to HLA-I ($p = 0.014$), and lower WBC ($p = 0.004$), neutrophil ($p = 0.066$), lymphocyte ($p = 0.012$), and eosinophil counts ($p = 0.022$; $p = 0.013$ for <250 cells/mm³; Table 4). The presence of allergies was not associated with ACR or AMR.

Because younger age at transplant is associated with a higher eosinophil count, allergies, and a potentially lower risk of rejection, we performed multivariable analysis that included age as a covariate. A lower eosinophil count as a continuous variable ($p = 0.010$) and female sex ($p = 0.009$) met statistical significance for ACR (Table 5A). In the multivariable analysis of risk factors for AMR (Table 5B), only the presence of PRA against HLA-I ($p = 0.014$) and a lower eosinophil count ($p = 0.011$) were statistically significant. By plotting the median eosinophil count with the probability of rejection by Loess curves, an inflection point illustrating an increase in the probability of ACR and AMR occurred at a cell count of 250 cells/mm³ (Figure 2A and B).

Given the existence of missing values in PRAs and DSAs ($n = 9$) and the potential confounding among the different leukocyte components with the eosinophil count, we examined the latter's relationship with AMR in a separate logistical model that only included the components of the

Table 2 Hematologic Profile of the Population Studied

Variables ^a	Total study population	Study population below 2.5 percentile of healthy children (%) ¹⁹	Age at transplant		p-value ^b
			< 3 years (n = 52)	> 3 years (n = 34)	
WBCs, K/mm ³	6.8 ± 1.9	8.1	7.3 ± 2.0	6.0 ± 1.4	0.002
Platelets, K/mm ³	282.0 ± 84.2	40.7	307.9 ± 86.6	242.5 ± 63.1	<0.001
Hematocrit, %	35.0 ± 3.9	37.2	33.9 ± 3.4	36.7 ± 4.0	0.001
Hemoglobin, g/dl	11.9 ± 2.0	24.4	11.4 ± 1.4	12.7 ± 2.6	0.002
MCV, fL	81.7 ± 5.6	2.3	80.9 ± 4.8	83.0 ± 6.6	0.096
RDW, %	14.4 ± 2.1	NA	14.6 ± 1.6	14.2 ± 2.7	0.288
Absolute neutrophil count, /mm ³	3,783 ± 1,292	0	3,893 ± 1,389	3,616 ± 1,128	0.333
POLYS, %	54.6 ± 9.9	NA	52.1 ± 9.3	58.5 ± 9.7	0.003
Absolute lymphocyte count, /mm ³	1,835 ± 796	14.0	2,034 ± 838	1,531 ± 624	0.004
Lymphocytes, %	28.3 ± 9.5	NA	29.3 ± 9.5	26.8 ± 9.3	0.241
Absolute monocyte count, /mm ³	637 ± 258	NA	717 ± 279	516 ± 159	<0.001
Monocytes, %	10.0 ± 3.2	NA	10.5 ± 3.5	9.2 ± 2.5	0.070
Absolute eosinophil count, /mm ³	195 (120–344)	NA	245 (143–498)	143 (100–207)	0.002
Eosinophils, %	3.0 (2.2–5.0)	NA	3.9 (2.6–6.9)	2.9 (1.9–3.8)	0.015
Absolute basophil count, /mm ³	20 (9–33)	NA	23 (9–45)	19 (8–24)	0.049
Basophils, %	0.4 (0.2–0.6)	NA	0.4 (0.2–0.8)	0.4 (0.2–0.5)	0.549

MCV, mean corpuscular volume; NA, reference value not available for the healthy population; POLYS, polymorphonuclear leukocytes; RDW, red blood cell distribution width.

^aContinuous variables are shown as mean ± standard deviation or as median (interquartile range) for skewed distributions.

^bThe p-values are based on the Student's *t*-test or the Mann-Whitney *U* test for variables with a skewed distribution of values.

leukocyte count without missing values (Table 5C). The only leukocyte component that was statistically significant was again the eosinophil count ($p = 0.018$).

Because the treatment for AMR was aggressive and typically included the use of ATG, which can affect leukocyte counts, we also performed a stratified analysis of all available CBC values before and after treatment of AMR to determine if the low eosinophil count associated with AMR was secondary to the AMR treatment itself. CBCs were available before treatment in 10 of the 11 patients with AMR. The white cell and absolute neutrophil counts decreased after treatment of AMR. However, lymphocyte and eosinophil counts were low even before treatment, with the eosinophil count increasing from 28 cells/mm³ (IQR 0–136 cells/mm³; $n = 608$,) to 42 cells/

mm³ (IQR 0–102 cells/mm³, $p = 0.055$; $n = 597$) after treatment. Lastly, no patient with AMR had a longitudinal eosinophil count > 250 cells/mm³ compared with 37 of 75 patients (49%) without AMR ($p = 0.020$) in the CBC analysis every 6 month (Table 6).

Discussion

In this study, we have shown significant CBC abnormalities in the pediatric heart transplant population, consistent with prior studies^{20,21} Moreover, our data suggest that a higher eosinophil count is associated with freedom from ACR or AMR. The association of the eosinophil count with freedom from rejection appeared to be independent of age, use of ATG for AMR, or other components of the CBC.

Previous reports, although limited, have observed that allergies are common among pediatric heart recipients,^{16,17} and our results confirm that observation. An elevated eosinophil count was associated with allergic conditions and allergic conditions were associated with transplant at a younger age; however, allergic conditions and younger age at transplant were not associated with freedom from rejection in our analysis. It is possible that allergies are too common, particularly in this population (56.3%), to be a useful predictor. Another possibility is that a certain immunologic state, manifested by a higher eosinophil count that is distinct from allergies, is involved in maintaining immunologic quiescence. One such possible state is the Th2 dominance over Th1, and an elevated eosinophil count may be one of its cellular biomarkers.

Indeed, increased eosinophils in the peripheral blood are associated with Th2-mediated cellular responses, such as those involved in allergic conditions,^{22–26} and

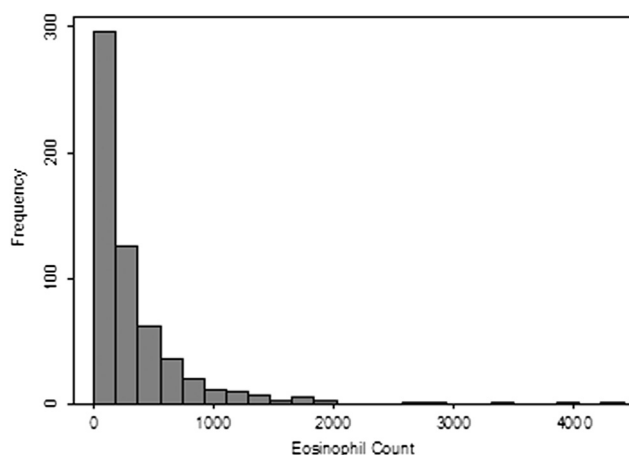


Figure 1 Histogram illustrates the distribution of the absolute eosinophil count in cells/mm³ in the entire study population.

Table 3 Univariate Analysis of Acute Cellular Rejection

Covariate ^a	No.	History of ACR (n = 38)	No ACR (n = 48)	p value ^b
Age at transplant, months		22.5 (9.75–123.50)	10.5 (4.25–109.75)	0.115
Age at transplant				
> 3 years	34	17 (45)	17 (35)	0.56
< 3 years	52	21 (55)	31 (65)	
Sex				
Female	42	23 (61)	19 (40)	0.082
Male	44	15 (39)	29 (60)	
Cardiac diagnosis				
Cardiomyopathy	35	19 (50)	16 (33)	0.129
Congenital heart disease	51	19 (50)	32 (67)	
Allergic condition				
Yes	49	24 (63)	25 (52)	0.382
No	37	14 (37)	23 (48)	
WBCs, K/mm ³		6.72 ± 2.00	6.81 ± 1.88	0.819
Counts, /mm ³				
Absolute neutrophils		3,855 ± 1,422	3,727 ± 1,190	0.651
Absolute leukocytes		1,773 ± 775	1,885 ± 817	0.518
Monocytes		630 ± 252	643 ± 265	0.824
Eosinophils		177 (98–327)	213 (133–432)	0.011
Basophils		21 (11–27)	20 (5–40)	0.102
ICFA, ATP ng/mL	56	357 (246–463) n = 30	351 (292–434) n = 26	0.712
WBC, mean/mm ³				
> 5,000	70	32 (84)	38 (79)	0.59
< 5,000	16	6 (16)	10 (21)	
Eosinophil count, median/mm ³				
> 400	16	4 (11)	12 (25)	0.102
< 400	70	34 (89)	36 (75)	
> 250	29	10 (26)	19 (40)	0.253
< 250	57	28 (74)	29 (60)	
PRA HLA-I				
> 10%	16	8 (22)	8 (18)	0.783
< 10%	65	29 (78)	36 (82)	
PRA HLA-II				
> 10%	14	7 (19)	7 (16)	0.774
< 10%	67	30 (81)	37 (84)	
DSA HLA-I > 5,000 MFI				
> 50	5	2 (6)	3 (7)	1
< 50	72	32 (94)	40 (93)	
DSA HLA-II > 5,000 MFI				
> 5,000	17	9 (26)	8 (19)	0.423
< 5,000	60	25 (74)	35 (81)	

ACR, acute cellular rejection; ATP, adenosine 5'-triphosphate; DSA, donor specific antibodies; HLA, human leukocyte antigen; ICFA, immune cell function assay; MFI, mean fluorescence intensity; PRA, panel reactive antibodies.

^aCategorical data are shown as number of patients (%), and continuous values are shown as mean ± standard deviation or median (interquartile range).

^bThe p-values are based on the chi-square test for categorical variables, the Student's t-test for continuous normally distributed variables, or the Mann-Whitney U test for non-normally distributed variables.

Th2-associated cytokines, IL-4, -5, and -10 have been shown to be important in achieving allograft tolerance after transplantation.^{27,28} Evidence from pediatric liver transplant patients demonstrated that those with a predominant Th2 cytokine profile had less ACR than those with Th1 phenotype.⁶ In vivo experiments involving renal transplant patients have shown that T cells cloned from patients with chronic rejection produce interferon-γ (a Th1 cytokine), whereas T cells of patients who are not rejecting produce IL-10 (a Th2 cytokine).²⁹

In addition to the balance between the Th1 and Th2 phenotypes, Tregs may be important in immunologic quiescence.^{8,9,30} Th2-associated cytokines induce Treg proliferation, which in turn suppresses rejection.^{10,11} Stenard et al³¹ demonstrated the potential benefit of monitoring peripheral Tregs in pediatric liver transplant patients by showing that levels of Treg cells were significantly lower during episodes of rejection. No previous study, however, has examined peripheral eosinophil count as a marker of Th2 or Treg dominance or their associations with freedom from

Table 4 Univariate Analysis of Risk Factors for Antibody Mediated Rejection

Covariate ^a	No.	History of AMR (n = 11)	No AMR (n = 75)	p-value ^b
Age at transplant, months		23.0 (11.00–145.00)	14.0 (5.00–110.00)	0.174
Age at transplant				1
> 3 years	34	4 (36)	30 (40)	
≤ 3 years	52	7 (64)	45 (60)	
Sex				0.755
Female	42	6 (55)	36 (48)	
Male	44	5 (45)	39 (52)	
Cardiac diagnosis				0.513
Cardiomyopathy	35	3 (27)	32 (43)	
Congenital heart disease	51	8 (73)	43 (57)	
Allergic condition				0.338
Yes	49	8 (72)	41 (55)	
No	37	3 (27)	34 (45)	
WBCs, K/mm ³		5.23 ± 1.54	7.00 ± 1.86	0.004
Counts, /mm ³				
Absolute neutrophils		3,116 ± 1,120	3,881 ± 1,292	0.066
Absolute leukocytes		1,280 ± 904	1,916 ± 752	0.012
Monocytes		556 ± 265	649 ± 256	0.269
Eosinophils		94 (62–160)	212 (133–364)	0.022
Basophils		19 (5–24)	21 (9–34)	0.136
ICFA ATP, ng/ml	56	304 (209–384) n = 9	356 (297–462) n = 47	0.274
WBCs, mean				<0.001
> 5,000 (70)	70	4 (36)	66 (88)	
≤ 5,000 (16)	16	7 (64)	9 (12)	
Eosinophil count, median				0.116
> 400	16	0 (0)	16 (21)	
≤ 400	70	11 (100)	59 (79)	
250	29	0 (0)	29 (39)	0.013
≤ 250	57	11 (100)	46 (61)	
PRA HLA-I				0.006
> 10%	16	6 (55)	10 (14)	
≤ 10%	65	5 (45)	60 (86)	
PRA HLA-II				0.019
> 10%	14	5 (45)	9 (13)	
≤ 10%	67	6 (55)	61 (87)	
DSA HLA-I > 5,000 MFI				0.014
> 5,000	5	3 (30)	2 (3)	
≤ 5,000	72	7 (70)	65 (97)	
DSA HLA-II > 5,000 MFI				0.214
> 5,000	17	4 (40)	13 (19)	
≤ 5,000	60	6 (60)	54 (81)	

AMR, antibody-mediated rejection; DSA, donor-specific antibodies; HLA, human leukocyte antigen; ICFA, immune cell function assay; MFI, mean fluorescence intensity; PRA, panel reactive antibodies.

^aCategorical data are shown as number of patients (%), and continuous values are shown as mean ± standard deviation or median (interquartile range).

^bThe p-values are based on the chi-square test for categorical variables, the Student's t-test for continuous normally distributed variables, or the Mann-Whitney U test for non-normally distributed variables.

Table 5 (A) Backward Stepwise Logistic Regression Analysis of Risk Factors for Acute Cellular Rejection^a

Clinical variable	Odds ratio	95% CI	p-value
Female sex	4.16	1.44–12.06	0.009
Age at Transplant	1.00	1.00–1.01	0.331
Ln median absolute eosinophil count	0.36	0.17–0.78	0.010

CI, confidence interval; Ln, natural logarithm.

^aIn addition to the covariates listed, the analysis also included sex, presence of allergic condition, mean white blood cell count, absolute neutrophil count, absolute leukocyte count, monocyte count, basophil count. Sample size of 86 with no missing data.

Table 5 (B) Backward Stepwise Logistic Regression Analysis of Risk Factors for Antibody-Mediated Rejection^a

Clinical variable	Odds ratio	95% CI	p-value
Age at transplant	1.00	0.99-1.01	0.891
PRA HLA class I > 10%	11.54	1.63-81.54	0.014
Ln median absolute eosinophil count	0.08	0.01-0.57	0.011

CI, confidence interval; DSA, donor-specific antibody; HLA, human leukocyte antigen; Ln, natural logarithm; MFI, mean fluorescence intensity; PRA, panel reactive antibody.

^aIn addition to the covariates listed, the analysis also included sex, presence of allergic condition, mean white blood cell count, absolute neutrophil count, absolute leukocyte count, monocyte count, basophil count, PRA HLA class II > 10%, DSA HLA-I > 5,000 MFI, DSA HLA-II > 5,000 MFI. Sample size of 77 (9 patients missing PRA or DSA).

Table 5 (C) Logistic Regression Analysis of Leukocyte Components as Predictors of Antibody-Mediated Rejection^a

Clinical variable	Odds ratio	95% CI	p-value
Mean white blood cell count	1.03	0.11-11.13	0.977
Mean absolute neutrophil count	0.84	0.21-3.32	0.804
Mean absolute leukocyte count	0.92	0.69-1.22	0.560
Median absolute eosinophil count	0.15	0.03-0.72	0.018

CI, confidence interval.

^aThe units for individual leukocyte counts are white blood cells/500, absolute neutrophil count/100, and absolute leukocyte count/100. Sample size of 86 with no missing data.

rejection in transplant recipients. Whether a higher eosinophil count itself is a biomarker of Th2 dominance, an epiphenomenon, or is directly involved in the pathway of protection from rejection, cannot be determined from this study, but the potential link to the Th1/Th2 paradigm is intriguing.

Earlier studies have observed a high eosinophil count at the time of ACR in adult renal, liver, pulmonary, and

cardiac transplant patients.³²⁻³⁵ Nagral et al³⁶ showed that an eosinophil count > 400/mm³ could predict rejection beginning 2 days before biopsy. These studies, however, used the maximum eosinophil count, focusing on an acute rise in the eosinophil count at the time of ACR, rather than examining the “average” eosinophil counts longitudinally. Hence, their results may reflect recruitment of eosinophils when rejection is already in process, which is different from our study examining an underlying immunological state of quiescence vs rejection and not the absolute eosinophil count at the time of rejection or quiescence.

In addition to a low eosinophil count, our study identified other factors, such as female sex (ACR) and the presence of HLA antibodies (AMR), associated with rejection, both of which are consistent with previous reports.^{37,38} With regard to the lack of association between rejection and age, it must be noted that many of the younger patients who received transplants at our center had complex congenital heart disease with a history of palliative repair, and therefore, exposure to immunologic stimulation early in life.

A conceivable explanation for the association of a low eosinophil count with AMR is that the ATG used to treat AMR can lower the eosinophil count. The eosinophil count in those treated for AMR in this study, however, was low before treatment. Furthermore, in those with ACR, of whom only 21% received ATG, there was also the suggested association of a higher eosinophil count with freedom from rejection.

Finally, it is important to note that the literature focuses on risk factors for rejection, not factors that are protective of rejection beyond demographics such as age. Although long-term adverse effects from immunosuppression in children can be deleterious, rejection remains a significant challenge in transplantation, particularly when AMR and late-onset ACR can have serious clinical sequelae.^{1,39,40} A reliable and easily monitored biomarker for protection from rejection, such as an eosinophil count, could potentially help guide surveillance and immunosuppression management. It is important to note that a higher eosinophil count was associated with a history of never having had rejection, as opposed to fewer rejections. Hence, the utility of this finding

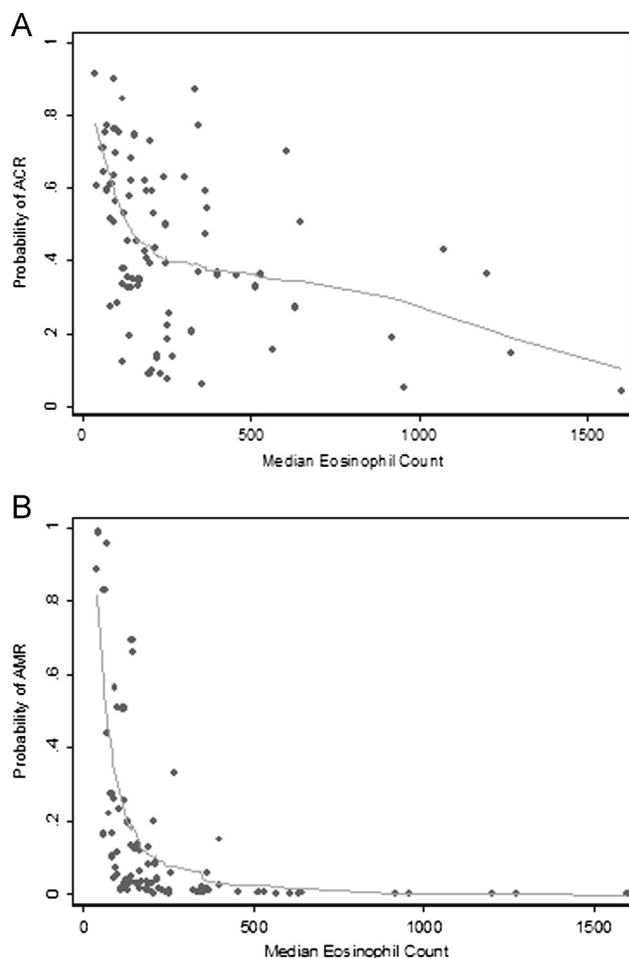


Figure 2 The probability of (A) acute cellular rejection (ACR) or (B) antibody-mediated rejection (AMR) and the absolute eosinophil count in cells/mm³.

Table 6 Summary of Characteristics of Patients With Antibody-Mediated Rejection

Patient No.	Age at transplant (months)	Eosinophil count (median/mm ³)	PRA		DSA	
			Class I	Class II	Class I	Class II
13	27	193 [130,246]	+	–	+	+
22	11	62 [20,697]	+	+	+	–
23	12	144 [24,292]	+	+	–	–
26	209	94 [74,113]	+	+	+	+
33	156	187 [167,208]	–	–	+	+
48	12	38 [24,68]	–	–	+	+
49	11	83 [42,124]	–	–	–	–
50	111	42 [31,70]	+	+	+	+
64	10	72 [56,87]	+	+	–	+
68	145	160 [147,211]	+	+	+	–
86	23	120 [119,140]	–	–	–	+

DSA, donor specific antibodies; PRA, panel reactive antibodies.

may allow children with a trend of higher eosinophil counts, such as a median of >250 cells/mm³, to be on lower immunosuppression after taking other clinical factors into consideration. Conversely, children with a lower eosinophil count may require more vigilante surveillance or even more maintenance immunosuppression if rejection occurs.

This study has some limitations. The study population had an adequate intermediate-term follow-up and was of good size for a pediatric cohort, but a larger sample size that contains more rejection episodes would strengthen the analysis. There is also an inherent variation in peripheral blood counts, both in the actual numbers circulating and in the counting method. We attempted to account for this variation by averaging longitudinal samples from individuals, and with numerous samples contributing to each patient's average value, this variation should be lessened. The differences in CBC values observed based on the age of 3 years at transplant (Table 2) do not take into account that these values can change over time in a longitudinal study because of aging and time out from transplant; neither of these variables were accounted for by the age at transplant in our analysis.

In conclusion, our results confirm the generalized bone marrow suppression and the high prevalence of allergic conditions seen in pediatric heart recipients and suggest that a high eosinophil count may confer a protective advantage against the development of ACR or AMR. Although many traditional risk factors exist to aid the transplant physician in profiling the patient at high risk for rejection, identifiers of low rejection risk in the heart transplant population are uncommon. If larger studies substantiate this finding, it will help minimize the burden of over-immunosuppression. Conversely, those with chronically low eosinophil count may require more careful adjustment of immunosuppression and surveillance. Future studies with a multi-institutional cohort will allow a better determination of the accuracy and utility of using eosinophil count to predict rejection risk. Such studies may also include a mechanistic arm to examine the T-cell phenotype and the Th1/Th2 balance in relation to the role of the peripheral blood eosinophil count and rejection risk.

Disclosures

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose.

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