ELSEVIER

The Journal of Heart and Lung Transplantation

http://www.jhltonline.org

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

Kate S. Arbon, BS,[a] Erin Albers, MD,[b] Mariska Kemna, MD,[b] Sabrina Law, MBBS,[c] and Yuk Law, MD[b]

From the [a]School of Medicine; [b]Department of Pediatrics, Seattle Children's Hospital, University of Washington, Seattle, Washington; and the [c]Department of Pediatrics, Morgan Stanly Children's Hospital, Columbia University, New York, New York.

KEYWORDS:
pediatric heart transplantation;
allergies;
rejection;
T helper phenotypes and rejection

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.

J Heart Lung Transplant 2015;34:1103–1111

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

Reprint requests: Yuk Law, MD, Department of Pediatrics, Seattle Children's Hospital, 4800 Sand Point Way, Seattle, WA 98115. Telephone: 206-987-1417.

E-mail address: yuk.law@seattlechildrens.org

1053-2498/$ - see front matter
http://dx.doi.org/10.1016/j.healun.2015.03.014

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 $\geq$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). Categoric 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 categoric 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