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TITLE
Allergies and Autoimmune Disorders in Children after Heart Transplantation

RUNNING TITLE
Allergies in Pediatric Heart Transplantation

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The authors do not have any conflicts of interest to disclose.

ABSTRACT
Pediatric heart transplantation requires lifelong immune suppression and may require thymectomy, both of which alter T-cell repertoires. We hypothesized that atopic and autoimmune diseases are more common in pediatric heart transplant patients than the general population, and that transplantation in early childhood increases the risk of development or worsening of atopic or autoimmune disease.

A cross-sectional single-centre study including 21 heart transplant patients aged ≤ 18 years was conducted. Data collected included age at transplant, induction, thymectomy, and development and severity of atopic or autoimmune disease. A majority (67%) reported having any atopic disease post-transplant, all of whom reported onset or worsening post-transplantation. Thymectomized patients were significantly more likely to have asthma (p=0.018) and report asthma worsening post-transplant (p=0.045). Patients with worsening of asthma post-transplant were transplanted at a significantly younger age (p=0.040). ABO incompatible and ABO compatible recipients presented similarly. Anemia was common (38%) but not always clearly of autoimmune origin.

Atopic diseases are common in children following heart transplantation: compared to the general population, there is a higher prevalence of eczema (43% versus 11%) and asthma (33% versus 9%). Both thymectomy and younger age at transplant are associated with atopic disorders, possibly due to altered T-cell repertoires.

KEYWORDS

Pediatric heart transplantation, allergies, autoimmune disorders, immune suppression, induction, thymectomy

INTRODUCTION

Heart transplantation is a life-saving intervention in infants and children with end-stage heart failure or congenital heart disease without promising surgical treatment. In order to prevent rejection, patients who have undergone cardiac transplantation require life-long suppression of the immune system. This may include intense induction therapy at the time of transplant with polyclonal anti-lymphocyte antibodies (anti-thymocyte globulin, ATG), resulting in subtotal depletion of lymphocytes, including mature and memory T- and B-cells. In the course of transplant or pre-transplant surgeries, incidental thymectomy to improve exposure of the operating field is commonly performed. The thymus is an essential organ for the maturation of bone-marrow derived precursor cells into a diverse pool of new effector and regulatory T-lymphocytes, and is a key structure in the development of self-tolerance. The combination of T-cell depletion by induction and removal of a key organ for regeneration of

these cells may have profound impact on the recipient's immunological competence and ability to differentiate self from foreign. A role of age and maturity of the immune system on incidence, prevalence, and type of manifestation of autoimmune and allergic disorders has previously been recognized in children with healthy immune systems. Accordingly, the timing of manipulation of the immune system via transplantation and immune suppression may influence the development of allergic and autoimmune disorders.

Allergic diseases such as allergic rhinoconjunctivitis, asthma, and atopic dermatitis have repeatedly been observed in patients after solid organ transplantation. In pediatric kidney, liver, and lung transplant recipients, the prevalence of type I sensitization to allergens was found in comparable numbers to the general population regardless of the type of transplanted organ [1,2,3,4]. There are a number of case reports of pediatric patients developing food allergies after solid organ transplantation, particularly in patients following liver transplantation versus any other solid organ transplant [5,6,7]. However, our clinical experience indicates that allergic manifestations are very common in heart transplanted children.

A number of studies have shown that thymectomy in infants and older children results in alterations of the peripheral T-cell compartment, including CD4+ and CD8+ T-lymphocytes [8,9] and regulatory T-cells [10]. The most prominent alterations seem to come with young age and early sampling post-thymectomy [11]. Thymectomy also results in premature senescence of the T-cell segment reflected in lower proportions of recent thymic emigrant cells and decreased levels of T-cell receptor excision circles (TREC), a marker for thymopoiesis in the blood [9,12]. The combination with T-cell depleting induction likely enhances these effects [13,14,15].

We hypothesized that the combination of removal of the thymus in cardiac transplant recipients, along with the treatment of T-cell depleting agents results in significant immune dysregulation, thereby increasing the likelihood of allergic and autoimmune disorders post-transplantation. With this study,

we sought to determine the incidence of these disorders in our patients and the role of potential additional risk factors such as thymectomy and younger age at transplant.

MATERIALS AND METHODS

We conducted a cross-sectional study of pediatric heart transplant patients aged ≤ 18 years seen at the Stollery Children's Hospital between 2013 and 2015. Institutional ethics approval was obtained. All pediatric heart transplant recipients were offered participation in the study, and consent and assent were obtained by the legal guardian and patient, respectively. Data were collected by (1) review of medical records and (2) standardized face-to-face or telephone survey. Data collected included demographics, underlying cardiac diagnosis, age at transplant, use and type of induction therapy, blood group (ABO) compatibility, development and severity of atopic or autoimmune disease, and family history of atopy and autoimmunity. Atopic or autoimmune disease was defined as being physician-diagnosed. Data obtained from patient interview was cross-referenced with medical records to ensure diagnostic accuracy. Allergic diseases, including but not limited to eczema, asthma, atopic urticaria, and food allergy were included; in addition to a variety of autoimmune diseases including type 1 diabetes mellitus and inflammatory bowel disease [Table 1]. Severity of disease was classified on a nominal scale from 0 to 3: 0 was defined as no diagnosis or problem, 1 was defined as "mild" disease leading to no impairment of activities, 2 was defined as "moderate" disease leading to some impairment (impairment of certain activities while still able to lead a normal life), and 3 was defined as "severe" with significant impairment to daily life. Removal of the thymus was documented in operative notes, although inconsistently documented ≤ 12 months of age. This is because removal as a whole was the standard approach of the cardiac surgeons for patients ≤ 12 months of age at our institution in the observed time frame; therefore thymectomy was presumed for transplants or pre-transplant cardiac surgeries occurring ≤ 12 months of age. Statistical analysis consisted of descriptive statistics, and comparison of dependent variables using the Fisher's exact test for categorical data and Mann-Whitney U test for quantitative data.

RESULTS

Out of 35 pediatric heart transplant patients meeting inclusion criteria, 21 were recruited into the study. Main reason for non-recruitment was unwillingness to participate in the study. Time from transplant date to study date was highly variable, averaging at 4.6 years. Demographics and clinical background are outlined in Table 2. None of the included patients had any detected genetic disorder affecting the immune system: including 22q11 microdeletion, trisomy 21, or congenital primary immunodeficiency. Indication for transplant was congenital heart disease in 11 patients (52%), and functional disease of an anatomically normal heart in 10 patients (48%) – including cardiomyopathy (9/21) and Kawasaki's disease (1/21). ATG induction was used in 14 patients (67%), while 3 received basiliximab and 3 received no induction. Decision around induction involved multiple considerations including age, immunological risk (HLA sensitization and ABO compatibility of the organ), and renal dysfunction. In terms of immunosuppressive maintenance therapy: as per standard protocol, patients received a combination of tacrolimus and mycophenolate mofetil, with prednisone used in high dose peri-transplant – weaned to 0.1 mg/kg daily by 21 days, and fully weaned off steroids by 3-6 months post-transplant. One patient (4.7%) was on sirolimus in place of tacrolimus due to progressive renal impairment. Four of 21 patients (19%) had received an ABO incompatible heart transplant, reflecting Edmonton as a pioneer centre for this novel approach.

Any type of atopic disease was present in the vast majority of patients (19/21, 90%) at any time point. A large proportion reported one or more atopic disease being present post-transplant (14/21, 67%), 100% of whom experienced the onset or worsening of the symptoms post-transplantation. Out of the 5 patients reporting one or more atopic disease at any time pre-transplant, 4/5 of these also had resolution of symptoms pre-transplant; only 1/5 reported persistence of symptoms to transplant (who also experienced resolution post). Most frequent were eczema (9/21, 43%), asthma (7/21, 33%), and non-viral urticaria (6/21, 29%). Rhinoconjunctivitis was reported in 3/21 (14%), while only 2/21 (9%) reported some form of food allergy including anaphylaxis [Figure 1]. Prevalence of eczema and asthma is pictorially compared to prevalence of these disorders in the general population [16] in

Figure 2. Severity of allergic symptoms were significant: of those with eczema, 45% rated the symptoms as being at least "moderate" or leading to some functional impairment. Similar proportions were obtained for those with asthma and urticaria: 42% rated symptoms of asthma being at least "moderate", while 33% of those with urticaria rated it as the same.

Within patients who had received thymectomy, 8/14 (57%) had asthma at any time while the remaining 43% reported no asthma. Of those who did not receive thymectomy, none (0/7) experienced asthma either pre-transplant or post-transplant. Post-transplant, of those who did receive thymectomy, 7/14 (50%) experienced new or worsening asthma. Patients who received thymectomy were significantly more likely to have asthma at any point in time (p=0.018) [Figure 3], and were significantly more likely to report worsening asthma symptoms post-transplant (p=0.045) [Figure 4].

Thymectomy was not significantly correlated with other atopic diseases post-transplant. Fisher's exact test for thymectomy versus new or worsening symptoms of other atopic diseases yielded the following results: p=0.642 for eczema, p=0.613 for atopic urticaria, p=1.000 for allergic rhinoconjunctivitis, p=0.533 for anaphylaxis, p=0.521 for non-anaphylactic food allergy.

In addition, patients transplanted at a younger age were significantly more likely to report worsening of asthma symptoms post-transplant (p=0.040 by Mann-Whitney U test) [Figure 5]. Age at transplant was not significantly correlated with the prevalence of other atopic diseases post-transplant. There was no association found between induction therapy and any type of atopic disease, and between ABO compatibility and atopic disease.

In regards to autoimmune diseases, 1 patient had biopsy-confirmed celiac disease and 1 patient eosinophilic esophagitis. No patients had type 1 diabetes, autoimmune thyroiditis, inflammatory bowel disease, or autoimmune joint disease. Statistical analysis was not performed for these diseases given the paucity of symptomatic subjects for analysis. Anemia was reported in 38% (8/21) post-transplant, although the anemia was not always clearly identified as being autoimmune in etiology.

Rather, its cause was often identified as multifactorial. ABO compatibility did not affect the rate of post-transplant anemia (p=1.000).

Family history of atopy and autoimmune disease in parents and/or siblings of subjects did not have any statistically significant effect on dependent variables: including prevalence of all types of atopy or autoimmune disease (p=0.367 for asthma).

DISCUSSION

Atopic diseases appear to be more common in pediatric patients following heart transplantation than in the general pediatric population. Based on *Statistics Canada*, prevalence of eczema and asthma in the general population are 11% and 9%, respectively [16]. Compared to the general population, there is a higher prevalence of eczema (43% versus 11%) and asthma (33% versus 9%) in our patients post-transplant. It is worth noting that 100% (14/14) of patients reporting any atopic disease being present post-transplant also reported the onset of symptoms or worsening of the disease following transplantation. Most commonly reported atopic conditions in our patients were eczema (43%) followed by asthma (33%). This proportional distribution of atopic disease is similar to what is observed in the general population: with eczema being more common that asthma, followed by other atopic conditions [16]. It is also worth noting that the severity of atopic symptoms was marked. Reporting severity of symptoms is a unique feature of the study, showing that a large proportion of the atopic symptoms in this population were rated as at least leading to some degree of functional impairment. Largely, these are conditions which cannot be ignored by our patient population, as they seem to be negatively impacting the patients while engaging in daily activities.

The origin of the increased prevalence of atopic disease seen in our patients is likely multifactorial. Interestingly, there was no significant correlation between family history of atopic disorders and the prevalence of atopy in our transplanted patients, albeit familial predisposition is one of the strongest risk factors in the general population [17]. This indicates that there may be acquired factors affecting

atopic risk in the context of transplantation, immune suppression, and thymectomy. Previous studies on patients with common variable immunodeficiency (CVID), a congenital B-cell deficiency that often only manifests later in childhood or young adulthood, have shown an increased frequency of allergic and autoimmune conditions with any being present in 38% of children [18]; however, these mostly presented as food intolerance, dermatologic conditions, and very rarely asthma. Similar observations were made in acquired immunodeficiency (AIDS) [19], suggesting that deficiency of parts of the immune response may result in overactivation of other pathways. Our study shows that thymectomy significantly increased the development or post-transplant worsening of atopic symptoms (mainly asthma). This is likely due to the alteration in T-cell repertoire, resulting in a smaller pool of naïve CD4+ T-cells and recent thymic emigrants. This hypothesis is supported by studies examining the effect of thymectomy on T-cell compartments in non-transplanted children [8,9], and in animal models [13,14]. Thymectomized individuals display lower levels of T-cell receptor excision circles (TREC), indicating a higher frequency of extrathymic differentiation of peripheral T-cells, and a limited variability pool of T-cells. Similarly, it is likely that regulatory T-cell repertoires remain limited, potentially leading to a deficit of internal immune regulation and resultant deinhibition towards self or harmless antigens.

Indeed, the thymus is a central lymphoid organ in humans. It plays a central role in the generation and maintenance of peripheral T-lymphocyte populations including those that have an indispensable role in the maintenance of self-tolerance and immune homeostasis. The regulation of the immune response is generated by a distinct population of T-lymphocytes termed regulatory T-lymphocytes (*Treg*) [20,21,22]. It is believed that the repertoire of *Treg* cells is selected by the recognition of self-antigens in the thymus, and is further shaped by self-antigen recognition in the periphery [22,23,24]. In animal models, there is evidence that the thymus is essential for the development and maintenance of *Tregs*, and that continued post-natal production of these regulatory cells is necessary to avert autoimmunity [25]. It is possible that this is a major cell type affected in our thymectomized transplant population. In fact, studies in young adults thymectomized during early childhood revealed a significant reduction in the diversity of the T-cell repertoire and accumulation of oligoclonal memory T-cells [8,9]. Further

to this, Halnon *et al* found that subjects with the most significant impairment in thymopoiesis most frequently reported symptoms suggestive of atopic dermatitis, for instance [10]. There is a limited number of studies that have investigated pediatric heart transplant recipients who have undergone surgical removal of the thymus and T-cell depletion. It was found that these subjects demonstrated nearly normal number of T-cells, but significantly restricted TCR diversity and a marked decrease in naive CD4+ T-cells and TREC levels. In these patients, T-cell dependent antibody response to common vaccines (diphtheria and tetanus) were significantly impaired [26]. Given the importance of thymic function demonstrated on T-cell repertoires, a future direction will be to examine T-lymphocyte subpopulations, including *Tregs*, in our patient population to potentially correlate with clinical data presented here.

We have further shown that age of transplant affected atopic symptoms, again notably with asthma. Younger age at transplant seems to be associated with increased development or worsening of atopic symptoms (mainly asthma), p=0.040. T-cell depletion with induction agents did not seem to affect atopy or autoimmunity in our study. Our results on age at transplant could be in keeping with aforementioned studies that have shown a greater decrease in naïve CD4+ T-cells with thymectomy at younger ages. We could consider this as being a greater impact to the developing immune system early in life, at a crucial time when immune tolerance is being created. Not only does younger age at transplant include earlier potential thymectomy, but also earlier immune suppressive medication. It is possible that the effect of immunosuppression is greater on the regulatory T-lymphocytes than other T-cell subpopulations early in life, and the addition of early thymectomy adds to this effect. No multivariate analyses were performed mindful of the small overall sample size; therefore, it was not possible to attribute the contributions of younger age at transplant and thymectomy independently. Although a statistically significant impact of ATG induction could not be shown in our study, we believe that the combination of early childhood thymectomy (resulting in impaired regenerative capacity and maturation of T-cells) and ATG induction (resulting in subtotal depletion of T-cell memory) result in a persistent immune dysbalance with deinhibition of autoimmune and allergic immune responses.

Interestingly, autoimmune disorders were not found to be significantly more common in post-transplant patients. There was also no significant correlation between family history of autoimmune disorders and the prevalence of these in our patients. It is unclear whether the relatively high prevalence of anemia reported (38%) was mostly due to autoimmunity. It was difficult to determine a single cause of anemia, given the presence of a variety of factors including: post-operative or iatrogenic blood loss, possible autoimmune hemolysis, and bone marrow suppression due to immunosuppressive medications (mycophenolate mofetil) or anti-infectious prophylaxes (such as valganciclovir and cotrimoxazole). Interestingly, we found that ABO incompatibility of the transplanted organ was not a significant risk factor for this. Other authors have described rare cases of autoimmune manifestations in pediatric solid organ transplant recipients, the majority of which presented as autoimmune cytopenias: including hemolytic anemia and idiopathic thrombocytopenia. These cases are predominant in liver transplant recipients, but have also been described following small bowel, kidney, and heart transplantation [27,28,29,30]. Autoimmune manifestations have further been attributed to preceding infections, namely EBV and CMV [28,31]. Additional phenomena such as de-novo autoimmune hepatitis after liver transplantation [32], and inflammatory bowel disease after solid organ transplantation [33] have been described.

ABO compatibility versus incompatibility did not play a role in the prevalence of anemia in our analysis, nor did it affect other autoimmune or atopic disorders. Compared to patient populations of other pediatric solid organ transplants, our cohort comprises of patients who have undergone cardiac transplantation predominantly as infants, preceded by at least one or more cardiothoracic surgeries (and incidental thymectomy) for congenital heart disease. Other authors have found that children who have received an ABO incompatible organ not only express fundamental differences in their immune response towards the blood group antigens [34], but also show major differences in B-cell memory and immune system response towards polysaccharide antigens [35,36]. These alterations were not associated with clinical differences in the prevalence of allergies and autoimmune disorders; however, larger cohorts need to be assessed to allow sufficiently powered conclusions.

Our study was limited by our small patient population, not allowing for highly powered statistical analyses. Extrapolation and applicability of this study to pediatric heart transplant patients as a whole is limited. Moving forward from this initial study, multi-centre recruitment will be essential. Moreover, since thymectomy is incidental and not performed as a therapeutic intervention, it is likely that the extent of thymus removal varied from patient to patient. Persistence of a small proportion of recent thymic emigrants in patient peripheral blood indicates that some thymic tissue typically remains. Another limitation is the lack of age-matched controls, which will be vital to examining larger numbers moving forward. Interviewer bias was avoided to the best of the study team's abilities. Consent bias may have been present since the main reason for non-recruitment was unwillingness to participate; however this bias and bias from misclassification were minimized by cross-referencing patient reported diagnoses with medical records to ensure accurate information. Objective measures of atopy – including skin prick testing, pulmonary function testing, and auto-antibody testing – would have been highly useful in avoiding bias from misclassification.

In summary, heart transplantation in infancy is associated with incidences of asthma and eczema post-transplant vastly exceeding the occurrence in the general population. Thymectomy and age at transplant play a role in the former, while family history appears to have less impact. In addition, ABO incompatibility was not associated to these disorders. Future studies should examine alterations in the immune phenotype and T-cell repertoire in this population. This may allow early identification of patients at risk, and may lead to modification of the immune suppressive strategies for these children. Avoidance or minimization of thymectomy during heart surgery or transplant may subsequently reduce the risk of atopic disorders. Awareness of the high frequency of these disorders in pediatric heart transplant recipients should result in regular clinical screening and early involvement of care specialists with initiation of specific therapies for these disorders.

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Disorder	At any time	New or worsened post-transplant	Severity	Triggers? Treatment?
Eczema / atopic dermatitis	Yes / No	Yes / No	0 / 1 / 2 / 3	
Urticaria (atopic)	Yes / No	Yes / No	0 / 1 / 2 / 3	
Rhinoconjunctivitis (atopic)	Yes / No	Yes / No	0 / 1 / 2 / 3	
Asthma	Yes / No	Yes / No	0 / 1 / 2 / 3	
Food allergy (anaphylaxis, allergy, EE †)	Yes / No	Yes / No	0 / 1 / 2 / 3	
Immune thrombocytopenic purpura	Yes / No	Yes / No	0 / 1 / 2 / 3	
Anemia (hemolytic)	Yes / No	Yes / No	0 / 1 / 2 / 3	
Autoimmune arthritis	Yes / No	Yes / No	0 / 1 / 2 / 3	
Diabetes mellitus type 1	Yes / No	Yes / No	0 / 1 / 2 / 3	
Autoimmune thyroiditis	Yes / No	Yes / No	0 / 1 / 2 / 3	
Celiac disease / EE † / IBD ‡	Yes / No	Yes / No	0 / 1 / 2 / 3	

Gender (N = 21)	
Female	12 (57%)
Male	9 (43%)
Diagnosis	
Structural heart disease	11 (52%)
Functional disease (normal anatomy)	10 (48%)
Age at transplant	
Median age (range)	2 years (10 days – 15 years)
≤1 year	9 (43%)
1 – 18 years	14 (67%)
Thymectomy	
Yes (transplant or other surgeries)	14 (67%)
ABO compatibility	
ABO compatible	17 (81%)
Induction therapy	
Anti-thymocyte globulin	14 (67%)
Basiliximab	3 (14%)
None	3 (14%)