Development of Human Leukocyte Antigen (HLA) Antibodies Against Vascular Homograft Donor in Pediatric Heart Transplant Recipients

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Background: The appearance of human leukocyte antigen (HLA) antibodies after solid organ transplantation predisposes recipients to graft dysfunction. In theory, vascular homografts, which are widely used in children with congenital heart defects, may cause allosensitization.

Material/Methods: In this single-center retrospective study, the presence of pre-existing HLA antibodies in pediatric heart transplant (HTx) recipients with a vascular homograft was evaluated in a cohort of 12 patients. HLA antibodies were screened before and after HTx and positive screening results were confirmed and identified using the Luminex® single antigen bead method. Endomyocardial biopsies (EMB) and coronary angiography studies were re-evaluated to assess the prevalence of acute rejections and coronary artery change in these patients.

Results: At the time of HTx, 8 patients (67%) had HLA antibodies detected by the Luminex assay, none of which were heart donor specific (DSA). All patients had negative leukocyte crossmatch. One patient developed DSAs against homograft donor prior to HTx. After the HTx, 5 patients (42%) developed DSAs against the heart donor and 4 patients (40%) against the homograft donor. In 2 patients (17%), the antibodies were against both heart and homograft donors. The rejection rate or prevalence of coronary artery vasculopathy did not differ significantly between the homograft cohort and our historical controls.

Conclusions: Our results suggest that the prevalence of DSAs against homograft donor prior to HTx is relatively rare. However, almost half of the patients developed DSAs against homograft post-HTx. The clinical importance of these antibodies warrants further studies.

MeSH Keywords: Antibody Formation • Cadaver • Child • Heart Transplantation • HLA Antigens • Vascular Grafting

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Background

Circulating donor-specific HLA antibodies (DSA) have been associated with increased risk for allograft vasculopathy and impaired graft survival in heart transplant (HTx) recipients [1–3]. The risk for developing DSAs is increased in patients with previous heart operations [4,5] and it has been suggested that pediatric recipients with congenital heart disease (CHD) have worse outcome than patients with cardiomyopathy [6,7]. Recent database analyses have established the presence of positive (>10%) panel reactive antibodies (PRA) before transplantation as a significant risk factor for 1-year mortality among pediatric HTx population [8].

The significance of HLA antibodies induced by vascular allografts and valve implants has remained unclear [9–12]. In patients with chronic kidney disease, vein allografts from deceased donors used for hemodialysis access have been suggested to lead to HLA sensitization [13], which may further cause humoral transplant rejection and graft dysfunction [14]. Vascular allografts have been used for decades among pediatric patients with congenital heart defects and it has been shown previously, that approximately 40% to 60% of these children develop HLA antibodies after allograft implantation [15–17]. Many of these patients with pre-existing HLA sensitization may require HTx during childhood or as adults. In the present study, we aimed to evaluate the presence of HLA antibodies against vascular homograft donor and heart transplant donor before and after HTx in a cohort of pediatric HTx patients with a vascular homograft from deceased donors. The long-term survival, number of acute rejections, and coronary arterial changes were evaluated retrospectively and compared to historical controls. We hypothesized that vascular homografts from deceased donors increases the risk for allosensitization before HTx which may further lead to increased number of rejections after HTx.

Material and Methods

Patients and patient selection

This is a single-center retrospective study of pediatric HTx recipients with a history of receiving vascular homografts from deceased donor at the Children's Hospital, Helsinki University Hospital (ethical approval no. HUS26/2018). Since 2000, HLA antibodies have been monitored systematically and the data were collected from all pediatric HTx patients transplanted between January 2000 and December 2015. A total of 44 patients underwent pediatric HTx. Seventeen patients had congenital heart disease (CHD), including hypoplastic left heart syndrome (HLHS) (n=6), univentricular heart (UVH) (n=3), transposition of the great arteries (TGA) (n=2), AV discordance (n=2), double outlet right ventricle (n=1), and pulmonary and/or tricuspid

atresia (n=2). All patients with CHD had palliative surgeries prior to HTx and in 12 cases (71%), vascular allografts from 18 deceased donors were used.

Vascular allografts were preserved in dimethyl sulfoxide (DMSO) cryopreservant (CryoSure-DMSO, WAK-Chemie) and stored in liquid nitrogen for a maximum of 5 years. Before use, the allografts were thawed according to the standard procedure. All vascular allografts were selected based on ABO blood grouping. The vast majority of the homografts consisted of pulmonary artery grafts; in 2 patients, aortic graft was used. No immunosuppressive medication was used during or after the operation.

The immunosuppressive protocol for all HTx patients consisted of anti-thymoglobulin induction therapy and triple medication including calcineurin inhibitor, azathioprine, and methylprednisolone (MP). None of the patients were put on immunosuppression after receiving of a vascular allograft from a deceased donor.

Data acquisition

The HLA antibody status, number of biopsy proven rejections, and coronary angiography findings were analyzed retrospectively from the data collected at routine follow-up visits at our center. The follow-up consists of anti-HLA antibody tests and endomyocardial biopsies (EMB) at 1, 3, 6, 9, 12, 18, and 24 months after HTx, and annually thereafter. Coronary angiography is performed annually in patients older than 4 to 5 years of age. The patients are transferred to adult care at the age of 18 to 22 years.

One Lambda Labscreen® mixed with Luminex® was used for HLA antibody screening and PRA calculation with the use of HLA Fusion software (One Lambda Inc., Canoga Park, CA, USA). In case of a positive screening result, the presence of HLA antibodies was confirmed and identified using the Luminex® single antigen bead method. A normalized MFI cutoff point of 1000 was used for positivity in single antigen analyses. Antibodies against HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ, and HLA-DP were analyzed. These results were compared to the HLA data of both vascular allograft donors and heart donors collected from the registry of the Finnish Red Cross Blood Service.

Endomyocardial biopsies (EMB) were classified according to the ISHLT criteria [18]. Grade 1R finding was defined as immunoactivation. Coronary angiograms were re-evaluated by an experienced pediatric cardiologist (JP). The angiography findings were classified according to the ISHLT guidelines into CAV grades 0–3 [19].

The Research Ethics Committee of Helsinki University Hospital approved the study; an informed written consent was obtained from all study subjects or their parents.

Table 1. Patients' demographics and characteristics. The table presents data from patients of the present study, from patients with congenital heart defect transplanted at our institution, and from all pediatric heart transplant patients at our unit. The data have been partly presented by Raissadati et al. [20].

	Present study (n=12)	Historical CHD (n=29)	Historical all (n=68)	p-Value*
Female/Male, %	50/50	42/58	53/47	0.873
Operations before HTx, n	3.4±1.2	3.0±1.4	1.4±1.5	1.00
Age at homograft insertion, years	4.0±6.3	NA	NA	
VAD, n (%)	0	0	6 (9)	1.00
Waiting time, days	125±210	168±223	150±201	0.353
Age at Htx, years	10.8±5.8	9.1±5.4	8.5±5.4	0.320
Follow-up time after HTx, years	5.6±4.7	13.2±8.0	12.7±7.5	0.009
Retransplantations, n (%)	2 (17)	3 (10)	5 (7)	0.620
HLA A/B match, n (%)	n=14			
0/4	2 (14)	3 (10)	9 (12)	0.620
1/4	10 (71)	12 (42)	31 (46)	0.165
2/4	2 (14)	7 (24)	16 (24)	0.702
3/4	0 (0)	0 (0)	4 (6)	
4/4	0 (0)	0 (0)	0 (0)	
HLA DR match, n (%)				
0/2	6 (43)	8 (28)	27 (40)	0.278
1/2	6 (43)	10 (34)	25 (37)	0.485
2/2	2 (14)	4 (14)	8 (12)	1.00
Mortality, n (%)	2 (17)	12 (41)	29 (43)	0.165
Acute cellular rejections				
Patients, n (%)	7 (67)	NA	NA	
Episodes, n	18	NA	103	
CAV at the time of the study	n=12	n=22	n=60	
CAV score 0, n (%)	4 (33)	12 (55)	38 (63)	0.410
CAV score 1, n (%)	2 (17)	2 (9)	4 (7)	0.602
CAV score 2, n (%)	3 (25)	2 (9)	9 (15)	0.391
CAV score 3, n (%)	0 (0)	4 (18)	4 (7)	0.273
NA	3 (25)	2 (9)	5 (8)	

CHD – congenital heart defect; HTx – heart transplant; CAV – coronary artery vasculopathy; CIT – cold ischemia time; VAD – ventricular assist device; NA – not available. Values are presented as mean ± standard deviation. * Comparison between present cohort and historical CHD patients.

Statistical analysis

Numerical data are presented as medians with ranges. The Mann-Whitney U test was used to compare patient groups with or without history of receiving homograft. Findings were considered statistically significant when the probability of a chance finding was less than 5% ($P<0.05$). SPSS version 22.0 software was used for statistical analysis.

Table 2. Clinical data in each individual patient.

Patient	Age at HTx	Homograft prior HTx, years	Cause of HTx	PRA class I/II,%	Match class I/II	CNI
1 M	6.6	1.4	AS	0/24	1/0	CyA>Tac
2 M	10.2	7.8	TOF	17/0	1/1	CyA>Tac
3 F	1.4	1.4	HLHS	61/87	1/0	CyA
4 F	6.4	5.9	HLHS	75/99	1/0	CyA>Tac
	15.7			57/66	1/0	Tac
5 F	10.1	9.7	HLHS	0/+	1/1	Tac
6 M	15.7	−0.1	TGA	0/0	2/2	Tac
7 M	16.2	16.2	HLHS	10/64	1/1	Tac
8 F	4.5	0.6	TGA	0/9	2/1	Tac
9 F	0.6	0.6	HLHS	52/45	0/0	Tac
10 M	15.3	14.9	HLHS	27/26	0/1	Tac
	16.1			0/0	1/2	Tac
11 M	19.2	0.0	HLHS	0/0	1/0	CyA
12 F	13.4	13.4	HLHS	74/99	1/1	Tac

M – Male; F – Female; HTx – heart transplantation; PRA – panel reactive antibody; CNI – calcineurin inhibitor; CyA – cyclosporine A; Tac – tacrolimusl CyA>Tac – patient switched from cyclosporine A to tacrolimus; AS – aortic valve stenosis; HLHS – hypoplastic left heart syndrome; TOF – tetralogy of Fallot; TGA – transposition of the great arteries.

Results

Patient demographics

Table 1 summarizes patient characteristics of the present study in respect to all pediatric HTx patients and patients transplanted due to CHD at our institution between 1991 and 2012 [20]. In the current patient cohort, a mean of 3.4 open heart surgeries had been performed prior to HTx. In 10 patients, vascular allografts from deceased donors were inserted a median of 1.37 (0.6–16.2) years prior to HTx. In 1 patient (Patient 11), a vascular homograft was inserted at HTx, and in another patient (Patient 6), 29 days after the HTx because of invasive *Candida albicans* infection of the aorta (Table 2). Vascular homografts from 18 different donors were used and HLA data were available from 16 donors (89%). Two patients had retransplantation and HLA type was available from all 14 heart donors. HLA antibodies were followed up according to our standard protocol and these data were available from all 12 patients. In 9 HTx patients, both heart and homograft donors had the same HLA type in a total of 16 alleles (class I, n=10 and class II, n=6).

HLA sensitization before HTx

Eight patients (67%) had HLA antibodies (PRA >10%) before HTx (Table 2). None of the pre-existing HLA antibodies were against heart donor, while 1 patient had DSA (DR1, MFI 2453) against the homograft donor. These homograft donor DSAs were detected for the first time 13.6 years after insertion of the homograft.

HLA sensitization after HTx

After HTx, a total of 7 patients (58%) developed anti-HLA antibodies. The median time from HTx to the first detection of DSAs was 69 (13–2865) days. In 5 patients (42%), the antibodies were *de novo* DSAs against heart donors and in 4 patients (40%), they were *de novo* DSAs against homograft donors. In 2 cases (Patient 4 and Patient 9), the post-HTx *de novo* DSAs were against both the heart and the homograft donor (Table 3). Patient 4 experienced biopsy-proven antibody-mediated rejection 2 weeks after the first HTx and developed severe coronary artery vasculopathy (CAV) later on. She was retransplanted 9 years after the first HTx. After the second HTx, she developed *de novo* DQ7 DSA against the heart and homograft donor (Table 3). In Patient 9, no coronary angiography or endomyocardial biopsy was performed because of her

antibodies in the development of CAV and significant graft dysfunction in 1 of the latter patients (Patient 4), however, the prevalence of biopsy proven rejections and CAV did not differ from historical controls. To the best of our knowledge, no previous study reporting the homograft donor DSAs after HTx has been published.

According to the previous studies, it is likely that exposure to the vascular allograft leads to humoral response and HLA immunization [15–17]. Meyers et al. have shown that the use of cryopreserved allograft tissue in the Norwood procedure is associated with a significant humoral response in the majority of patients, especially in those who were mismatched for HLA type [15]. The major concern with HLA antibodies is consequently prolonged waiting times for HTx, increased risk for antibody-mediated rejection due to pre-existing HLA antibodies, and dysfunction of the vascular allograft [21]. The data about the effect of homograft induced HLA sensitization on rejection rate and graft function after HTx are scarce. In pediatric heart transplant recipients, a PRA 10% or more was associated with increased mortality and rejection rate compared with those with a PRA under 10% [5]. Our retrospective analysis on biopsy-proven acute rejection and CAV rate did not reveal any statistically significant difference between the HTx recipients with vascular allograft and our transplant population in general. Our data do not support the initiation of immunosuppression for recipients of a vascular allograft from deceased donors.

The immunogenicity of the allograft depends on treatment and preservation of the graft. Vascular allograft treatment model that removes cellular elements leaving only the tissue matrix has been developed. These decellularization techniques have been shown to reduce the risk for homograft-induced sensitization compared to DMSO-treated grafts [22]. Cryopreserved grafts contain viable endothelial cells, which increases the risk for an immunogenic response [23,24]. Despite this recent development in the homograft treatment methods, there are hundreds of patients with grafts that were inserted in the 1990s when less effective decellularization methods were commonly used. In our cohort, 1 patient developed *de novo* DSA against the homograft donor more than 10 years after the operation, which should be taken into consideration when preparing patients for heart transplantation.

The major caveats of the study were the small sample size and retrospective nature. However, there were also strengths, such as systematic routine follow-up of HLA antibodies, EMBs, and coronary angiographies. Data regarding the HLA genotype of the homograft and heart donors were also available from nearly all participants.

Conclusions

We conclude that the risk for development of HLA antibodies prior to HTx is relatively small among patients with vascular allograft from deceased donor. Donor-specific HLA antibodies against homograft donor might appear after HTx. However, the clinical importance of this finding remains to be studied.

Conflicts of interest

None.

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