OPEN

Recurrent Mild Acute Rejections and Donor-specific Antibodies as Risk Factors for Cardiac Allograft Vasculopathy in a National Pediatric Heart Transplant Cohort

Anu K. Kaskinen, MD, PhD,[1] Juuso Tainio, MD, PhD,[1] Jaana I. Pihkala, MD, PhD,[2] Juha P. Peräsaari, PhD,[3] Jouni Lauronen, MD, PhD,[3] Alireza Raissadati, MD, PhD,[4] Jussi M. Merenmies, MD, PhD,[1] Hannu J. Jalanko, MD, PhD,[1] and Timo Jahnukainen, MD, PhD[1]

Background. Immune-mediated factors such as acute cellular rejections and donor-specific antibodies (DSAs) are risk factors for cardiac allograft vasculopathy (CAV). We studied a national cohort with a unified setting and thorough protocol endomyocardial biopsy (EMB) data for an association between cellular rejections, especially when mild and recurrent, and DSAs with CAV in pediatric heart transplant (HTx) patients. **Methods.** This is a retrospective, national cohort study of 94 pediatric HTxs performed between 1991 and 2019 and followed until December 31, 2020. Diagnosis of CAV was based on reevaluation of angiographies. Protocol and indication EMB findings with other patient data were collected from medical records. Associations between nonimmune and immune-mediated factors and CAV were analyzed with univariable and multivariable Cox regression analyses. **Results.** Angiographies performed on 76 patients revealed CAV in 23 patients (30%). Altogether 1138 EMBs (92% protocol biopsies) were performed on 78 patients (83%). During the first posttransplant year, grade 1 rejection (G1R) appeared in 45 patients (58%), and recurrent (≥2) G1R findings in 14 patients (18%). Pretransplant DSAs occurred in 13 patients (17%) and posttransplant DSAs in 37 patients (39%). In univariable analysis, pretransplant DSAs, appearance and recurrence of G1R findings, and total rejection score during the first posttransplant year, as well as recurrent G1R during follow-up, were all associated with CAV. In multivariable analysis, pretransplant DSAs and recurrent G1R during the first posttransplant year were found to be associated with CAV. **Conclusions.** Our results indicate that pretransplant DSA and recurrent G1R findings, especially during the first posttransplant year, are associated with CAV after pediatric HTx. (Transplantation Direct 2023;9: e1534; doi: 10.1097/TXD.0000000000001534.)

Chronic rejection in heart transplant (HTx) presents as cardiac allograft vasculopathy (CAV), manifesting as progressive and diffuse intimal proliferative arteriosclerosis.[1] CAV can cause late graft failure and death after pediatric HTx. Approximately half of pediatric HTx recipients develop CAV during the first 15 posttransplant years.[2] Recipient- and donor-related risk factors for CAV include general metabolic risk factors for atherosclerosis, cytomegalovirus infection, older donor or recipient age, stroke as donor cause of death, and graft cold ischemia time.[1] More importantly, immune-mediated factors such as acute cellular rejections and donor-specific antibodies (DSAs) lead to vascular endothelial inflammation and contribute to CAV development.[3]

Acute cellular rejection, especially if moderate to severe or associated with hemodynamic compromise, is a well-established risk factor for CAV both in adult and pediatric

Received 5 July 2023.

Accepted 24 July 2023.

[1] Department of Pediatric Nephrology and Transplantation, New Children's Hospital, Pediatric Research Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland.

[2] Department of Pediatric Cardiology, New Children's Hospital, Pediatric Research Center, Helsinki University Hospital and University of Helsinki, Helsinki, Finland.

[3] Finnish Red Cross Blood Service, Histocompatibility Laboratory, Helsinki, Finland.

[4] Division of Cardiology, Department of Pediatrics, Stanford School of Medicine, Helsinki, Finland.

Correspondence: Anu Kaskinen, MD, PhD, Department of Pediatric Nephrology and Transplantation, New Children's Hospital, Stenbäckinkatu 9, 00290 Helsinki, Finland. (anu.kaskinen@helsinki.fi); Twitter: @AnuKaskinen.

A.K.K., J.T., H.J.J., and T.J. participated in research design. A.K.K., J.T., J.I.P., J.P.P., J.L., A.R., and J.M.M. participated in data collection. A.K.K. participated in data analysis and acquisition of funding. A.K.K., J.T., J.I.P., J.P.P., J.L., A.R., J.M.M., H.J.J., and T.J. participated in the writing of the article.

The authors declare no conflicts of interest.

This work was funded by the Finnish Foundation for Pediatric Research.

http://links.lww.com/TXD/A568

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001534

populations.[2] Mild grade 1 rejections (G1Rs), which often are left untreated, may contribute to CAV, especially in case of recurrence.[4,5] Both pretransplant and de novo DSAs (dnDSAs), especially class II HLA DSAs, may predispose patients to CAV.[6,7] Today, data on these immune-mediated risk factors for CAV remain scarce in pediatric populations, and most studies have investigated only DSA or endomyocardial biopsy (EMB) findings in different study settings. Thus, we aimed to study whether cellular rejections, especially mild and recurrent, and DSAs increase CAV risk in our national cohort of 94 pediatric HTx patients.

PATIENTS AND METHODS

Patients

All Finnish pediatric HTxs were performed and followed up at least annually at Children's Hospital, Helsinki University Hospital, until 2018 and thereafter at New Children's Hospital. This study comprised a complete national cohort of 94 pediatric HTx recipients who received an HTx between 1991 and 2019. Patients were followed up until death, retransplantation, transition to adult care (approximately 20 y old), or the last observation day on December 31, 2020. All patient data, except angiographies, were collected by reviewing patient records. Clinical data on heart donors were collected from the organ allocation office's medical records. The institution's ethics committee approved the study protocol. For register-based research, no individual consent was required.

Immunosuppression

In all patients, triple immunosuppression consisted of cyclosporine A until 2012 or tacrolimus from 2013 onward, in addition to azathioprine and methylprednisolone. Methylprednisolone dosage was tapered in the first year after HTx. Antithymocyte globulin (ATG) was used for induction therapy. Our immunosuppression protocol has been detailed previously.[8]

EMBs and Rejections

In our institution, EMBs are performed in children aged ≥4 y. Protocol EMBs were performed 2 to 3 wk after HTx, approximately biweekly until discharge, then 3, 6, 9, 12, 18, and 24 mo after HTx, and then annually. Indication biopsies were performed in case of clinical rejection suspicion and on a case-by-case basis for controlling EMB findings 4 to 8 wk after initiation of rejection treatment. Biopsy findings were classified according to the 2004 International Society for Heart and Lung Transplantation (ISHLT) grading system and abstracted from medical records.[9] Patients with ≥2 episodes of G1R were classified as having recurrent G1R. We calculated the total rejection score (TRS), adapted from a previous study.[10] All EMBs were scored as G0R = 0, G1R = 1, G2R = 2, and G3R = 3, and scores were normalized by dividing by total number of EMBs taken during the period of interest. According to our protocol, ≥grade 2 rejections (G2Rs) and any grade symptomatic rejections were treated with methylprednisolone pulses of 10 mg/kg, whereas asymptomatic G1Rs were preferably treated with increased dose of oral methylprednisolone (1–2 mg/kg/d). Enhancement of maintenance immunosuppression was considered case by case. In the case of verified antibody-mediated rejection (AMR), plasmapheresis, ATG, or rituximab were introduced.

Coronary Angiographies

Protocol coronary angiography (CA) was performed in children aged ≥4 y after HTx and annually thereafter. Selective CA was the standard investigation, but in some children weighing <20 kg, coronary arteries were evaluated from aortic root angiograms. All CAs were reevaluated by a single cardiologist (J.P.) to grade the CAV severity according to 2010 ISHLT guidelines.[11]

HLA Typing, Cytotoxic Crossmatching, and DSA Analyses

HLA typing methods have changed over time. Since 2007, recipients were typed for low-resolution HLA-A, -B, and -DR with Luminex using One Lambda LABType kits, and since 2015, donors with LinkSēq HLA-ABCDRDQA1DQB1DP 384 Typing Kit (One Lambda Inc, West Hills, CA) according to manufacturers' instructions. For recipient typing, only serology was used until 1997. Between 1997 and 2007, DRB1 HLA was typed with sequence-based oligonucleotide probes (Inno-LiPA, Innogenetics, Ghent, Belgium). Before mid-2015, donors were HLA typed with sequence-specific primers.

The complement-dependent cytotoxicity crossmatches with the Amos technique were performed with purified T cells (Fluorobeads, One Lambda) and with density gradient–purified splenocytes until 2015. Immunodensity-purified peripheral blood T and B cells (RosetteSep, Stemcell Technologies, Vancouver, Canada) were used thereafter. Final crossmatch was considered positive if any individual T-cell or splenocyte/B-cell crossmatch was positive.

HLA antibody analyses were performed according to clinical surveillance after Luminex antibody screening began in 2006. The antibody status of stored samples beore 2006 was updated later. Donor specificity of all antibodies was evaluated, apart from HLA-DP antibodies because donor HLA-DP types were unknown in all donors typed before 2015. Sensitization was defined as calculated panel-reactive antibody measurement of ≥10% for either class I or II HLA. A mean fluorescence intensity (MFI) of >1000 was considered positive, and cumulative MFI was the MFI sum of all positive DSAs. We further classified patients with DSAs by the presence of persistent DSAs (presence of DSAs on ≥2 separate occasions for at least 2 y), transient DSAs (presence of DSAs on ≥2 separate occasions for <2 y), or single DSA (presence of DSAs only once).

Statistics

We compared qualitative variables (number of patients with percentages) with the χ^2 test and continuous variables (median with interquartile range [IQR], or mean ± SD, as appropriate) with the *t* test or Mann-Whitney *U* test, as appropriate. We estimated survival probabilities using the Kaplan-Meier method and compared them by log-rank analysis. Cox proportional hazard model was used for both univariable and multivariable regression analyses to estimate risk factors affecting CAV-free survival and graft survival. Variables with a *P* value of ≤0.1 (model 1) or *P* value of <0.05 (model 2) in univariable analyses were considered significant and included in multivariable models. Of the correlating factors, 1 was included in the multivariable model and selected on the basis of clinical reasoning. For analyses on CAV as an outcome, patients were excluded if their follow-up was <1 y and no angiographies performed (n = 18). The level of statistical significance was set at 0.05 for all analyses. Statistics were

analyzed using SPSS version 28.0 (SPSS, Inc., Chicago, IL) and Prism version 9.3.1 (GraphPad Software, La Jolla, CA).

RESULTS

Patient Characteristics and Outcome

Between 1991 and 2019, 94 patients underwent HTx. The median age at the time of HTx was 8.7 y (range, 0.47–19.49 y). Table 1 shows recipient and donor characteristics. At the end of follow-up, 36 patients had transitioned to adult care after a median pediatric follow-up of 8.1 (range, 1.6–18.9) y, 37 remained in pediatric follow-up, and 21 had either died (n = 18) or undergone re-HTx (n = 3). Five patients (5%) died within 30 d of operation, and a further 13 deaths (14%) occurred on average 3.4 y (range, 0.11–11.28 y) after HTx. Group 1 had more graft losses (43% versus 2%, $P = 0.003$) and lower left ventricular ejection fraction at the end of follow-up (61% versus 70%) than group 2 (Table 2, Figure 1).

TABLE 1.

Recipient and donor characteristics among patients who developed CAV (group 1) and those who did not (group 2)

	N = 94	Group 1, CAV (n = 23)	Group 2, no CAV (n = 53)	HR (95% CI)	P[a]
Recipient characteristics					
Age, y	8.7 (3.1–13.5)	7.4 (4.1–13.2)	11.3 (4.6–14.1)	1.05 (0.96–1.15)	0.29
Male gender	45 (48%)	10 (44%)	26 (49%)	1.03 (0.45–2.37)	0.94
Indication for transplantation					
Congenital heart defect	42 (45%)	11 (48%)	19 (36%)	1.72 (0.75–3.94)	0.20
Cardiomyopathy	50 (53%)	12 (52%)	34 (64%)		
Other[b]	2 (2%)	0 (0%)	0 (0%)		
Previous cardiothoracic surgery	55 (59%)	15 (65%)	26 (49%)	1.53 (0.65–3.61)	0.33
Positive CDC crossmatch	5 (5%)	4 (17%)	1 (2%)	2.26 (0.76–6.70)	0.14
HLA recipient–donor mismatch					
AB	3 (2–3)	3 (2–3)	3 (2–3)	1.18 (0.73–1.91)	0.50
DR	1 (1–2)	2 (1–2)	1 (1–2)	1.08 (0.54–2.14)	0.83
Sensitized before HTx[c]	16 (17%)	6 (38%)	5 (11%)	2.69 (0.98–7.44)	0.06
CPRA I pretransplant,[d] %	19 (0–69)	35 (0–94)	14 (1–39)	1.01 (1.00–1.03)	0.61
CPRA II pretransplant,[d] %	26 (0–69)	37 (1–94)	3 (0–52)	1.01 (1.00–1.03)	0.06
DSA pretransplant[e]	13 (17%)	7 (37%)	3 (6%)	3.78 (1.46–9.76)	0.006
CMV seropositivity	43 (46%)	10 (44%)	27 (51%)	0.95 (0.41–2.18)	0.91
CMV donor–recipient mismatch (D^+/R^-)	27 (29%)	6 (26%)	18 (34%)	0.65 (0.26–1.66)	0.37
Era (since 2005)	46 (49%)	28 (53%)	8 (35%)	0.90 (0.37–2.17)	0.81
ABO-incompatible HTx	4 (4%)	0 (0%)	2 (4%)	0.05 (0.00–12 133)	0.63
Graft cold ischemia, min	197 (151–241)	187 (146–260)	208 (156–254)	1.00 (1.00–1.00)	0.52
Donor characteristics					
Age, y	13.7 (4.8–29.8)	7.5 (5.6–17.6)	15.3 (7.3–30.1)	1.00 (0.97–1.04)	0.77
Male gender	44 (47%)	12 (52%)	25 (47%)	1.09 (0.47–2.51)	0.845
BMI,[f] kg/m^2	18.8 (16.0–21.4)	19.1 (16.5–23.1)	18.8 (16.5–21.4)	1.12 (1.00–1.27)	0.06
CMV seropositivity	54 (57%)	12 (52%)	35 (66%)	0.69 (0.30–1.56)	0.37
Stroke as cause of death	26 (28%)	3 (13%)	17 (32%)	0.47 (0.14–1.60)	0.23

Data are presented as n (%) or median (IQR), as appropriate.
[a]Effect of recipient and donor characteristics for CAV development analyzed by univariable Cox regression analysis.
[b]One total atrioventricular block and 1 thrombotic occlusion of left coronary ostial stenosis.
[c]CPRA data available for 71 patients, 16 in group 1 and 45 in group 2.
[d]Patients with CPRA >0% for either class I or II HLA included (n = 21).
[e]DSA data available for 78 patients, 19 in group 1 and 48 in group 2.
[f]Donor BMI data available for 75 patients, 18 in group 1 and 43 in group 2.
BMI, body mass index; CAV, cardiac allograft vasculopathy; CDC, complement-dependent cytotoxicity; CI, confidence interval; CMV, cytomegalovirus; CPRA, calculated panel-reactive antibody; DSA, donor-specific antibody; HR, hazard ratio; HTx, heart transplant; IQR, interquartile range.

TABLE 2.

Outcome after HTx among patients who developed CAV (group 1) and those who did not (group 2)

Outcome after HTx	All patients (N = 94)	Group 1, CAV (n = 23)	Group 2, no CAV (n = 53)	HR (95% CI)	P[a]
Graft loss (death or retransplantation)	21 (22%)	10 (43%)	1 (2%)	23.7 (3.03–185.87)	0.003[b]
LVEF at end of follow-up,[c] %	68 (60–75)	61 (54–67)	70 (63–75)		0.0006
Follow-up time,[d] y	5.9 (2.6–10.3)	6.3 (3.4–11.3)	6.0 (3.6–11.1)		0.76

Data are presented as n (%) or median (IQR), as appropriate.
[a]Comparisons between groups 1 and 2 by the Mann-Whitney *U* test unless otherwise stated.
[b]Effect of CAV for graft loss analyzed by univariable Cox regression analysis.
[c]n = 86.
[d]Intraoperative deaths during HTx operation excluded, n = 92.
CAV, cardiac allograft vasculopathy; CI, confidence interval; HR, hazard ratio; HTx, heart transplant; IQR, interquartile range; LVEF, left ventricular ejection fraction.

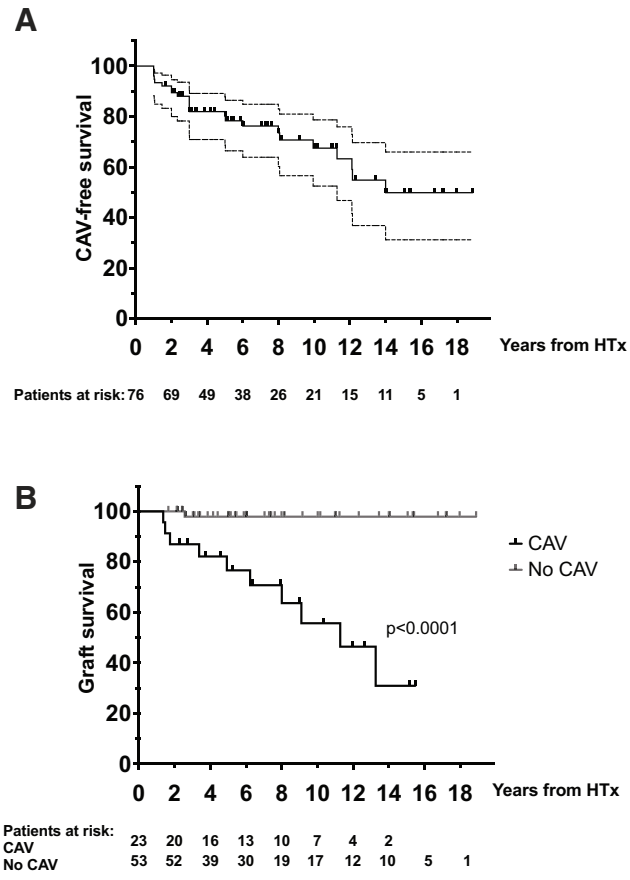

FIGURE 1. Kaplan-Meier estimated CAV-free survival (A) and log-rank comparison of Kaplan-Meier estimated graft survival (B) between patients who developed CAV and those who did not. CAV, cardiac allograft vasculopathy; HTx, heart transplant.

EMB and Anti-HLA Antibody Findings

Altogether 1138 EMBs were performed during the follow-up for 78 patients (83%) at a median of 15 (10–19) procedures; 91 EMBs were performed because of clinical indications for 40 patients (43%) after first posttransplant year. Biopsies were performed during the first 3 mo after HTx in 74 patients (79%), during the first posttransplant year in 77 patients (82%), after 1 y in 73 patients (78%), and after 3 y in 56 patients (60%; Table 3). Group 1 underwent more EMBs during the first 3 mo and first year after HTx than group 2 (Table 3).

Fifty-nine patients (76%) had a median of 2 (1–3) histologically proven rejection episodes during follow-up. G1R occurred in 54 patients (69%), G2R in 11 patients (12%), recurrent G1R in 36 patients (46%), and AMR in 6 patients (8%); no grade 3 rejection (G3R) occurred (Table 3). During the first posttransplant year, protocol EMBs revealed rejection in 46 patients (60%) and 1 patient showed G2R in an EMB taken because of clinical indication. After the first posttransplant year, protocol EMBs revealed rejection in 36 patients (50%), whereas indication EMBs revealed G1R findings in 3 patients (8%) and G2R findings in another 3 patients (8%). The median TRS showed no difference between first year after transplant (0.13 [0.00–0.25]) and thereafter (0.00 [0.07–0.23], P=0.94).

All rejections, regardless of grading, were treated during the first 3 mo posttransplant and later if clinical rejection was suspected. After 3 mo, all G2Rs and all rejection findings revealed by biopsies taken because of clinical rejection suspicion were treated. However, after 3 mo, at least some G1R findings revealed by protocol biopsies were left untreated in 26% of patients.

Pretransplant DSAs, occurred more often in group 1 than in group 2 (37% versus 6%; Table 1). Thirty-seven patients (39%) had a median of 2 (1–3) posttransplant DSAs and 34 (36%) patients developed dnDSAs (Table 4). Posttransplant DSAs developed a median of 0.09 (0.06–0.97) y after HTx. No difference in the appearance of posttransplant DSAs occurred between groups 1 and 2 (Table 4).

Cardiac Allograft Vasculopathy

Angiography was performed on 76 (81%) patients, of whom 23 (30%) developed CAV a median 3 y (1.47–8.1) after HTx (Figure 1). Severity of CAV was mild in 17 (74%) patients and moderate to severe in 5 (22%). In 1 patient, CAV was discovered at autopsy and angiographic degree of CAV could not be evaluated. Kaplan-Meier estimated survival of patients alive 1 y after transplant and diagnosed with CAV was 77% and 56% at 5 and 10 y after HTx, respectively, and poorer compared with patients without CAV (P<0.0001; Figure 1).

Risk Factors for CAV

Of the pre- and perioperative factors, only the presence of pretransplant DSAs was associated with increased CAV risk in univariable analysis (Table 1). Of the posttransplant EMB findings, the appearance of G1R findings during the first posttransplant year and of G2R findings within 3 mo after HTx was associated with CAV (Table 3). Recurrent G1R and TRS during the first year and recurrent G1R during the total follow-up were also associated with increased CAV risk (Table 3). However, the rejection findings after the first posttransplant year were not associated with CAV (Table 3).

Recurrent G1R during the first year after HTx and donor body mass index were independent significant risk factors for CAV development in multivariable analysis, including covariates with a P value of <0.1 in univariable analyses. The presence of pretransplant DSAs in addition to recurrent G1R during the first year after HTx remained independently significant risk factors for CAV development in multivariable analysis, including only covariates with a P value of <0.05 in univariable analysis (Table 5, Figure 2).

DISCUSSION

Despite advances in posttransplant management, CAV remains a significant challenge after pediatric HTx and contributes to fibrosis and impaired heart graft function.[1] The vascular endothelial inflammation leading to CAV is triggered by immune-mediated factors such as T cell–mediated and AMRs and DSAs.[1] In this national pediatric HTx cohort, pretransplant DSA and recurrent mild rejections during the first posttransplant year were risk factors for CAV. To the best of our knowledge, this is the first pediatric retrospective study using both DSAs and thorough protocol EMB data.

Almost a third of the patients were diagnosed with CAV, and Kaplan-Meier estimated CAV-free survival was 80%, 67%, and 50% at 5, 10, and 15 y after HTx, respectively. Estimated CAV incidence was slightly higher at 5 and 10 y in our cohort than in large registry data showing estimated CAV incidence of 13% to 15% at 5 y and 25% at 10 y.[2,12] However,

TABLE 3.

EMB findings after HTx among patients who developed CAV (group 1) and those who did not (group 2)

	All patients (N = 94)	Group 1, CAV (n = 23)	Group 2, no CAV (n = 53)	HR (95% CI)	P[a]
During follow-up	n = 78 (83%)	n = 23 (100%)	n = 53 (100%)		
No. EMBs per patient	15 (10–19)	17 (11–21)	14 (10–19)		0.27[b]
Grade 1	54 (69%)	20 (87%)	33 (62%)	3.1 (0.93–10.6)	0.07
Grade 2	11 (12%)	5 (22%)	6 (11%)	1.44 (0.53–3.89)	0.47
Recurrent grade 1	36 (46%)	17 (74%)	19 (36%)	3.91 (1.51–10.12)	0.005
AMR	6 (8%)	4 (17%)	2 (4%)	1.39 (0.46–4.22)	0.56
≤3 mo after HTx	n = 74 (79%)	n = 23 (100%)	n = 53 (100%)		
No. EMBs	5 (4–6)	6 (5–7)	4 (3–6)		0.02[b]
Grade 1	38 (51%)	14 (61%)	23 (43%)	1.76 (0.76–4.08)	0.19
Grade 2	5 (7%)	4 (17%)	1 (2%)	3.72 (1.23–11.26)	0.02
Recurrent grade 1	10 (14%)	6 (26%)	4 (8%)	2.56 (0.99–6.65)	0.05
Total rejection score	0.14 (0.00–0.25)	1.7 (0.00–0.50)	0.00 (0.00–0.25)	2.27 (0.50–10.21)	0.29
≤12 mo after HTx	n = 77 (82%)	n = 23 (100%)	n = 52 (98%)		
No. EMBs	7 (6–8)	8 (7–9)	6 (5–8)		0.03[b]
Grade 1	45 (58%)	17 (74%)	27 (51%)	2.62 (1.03–6.70)	0.04
Grade 2	6 (8%)	4 (17%)	2 (4%)	2.24 (0.76–6.61)	0.14
Recurrent grade 1	14 (18%)	8 (35%)	6 (11%)	3.31 (1.36–8.05)	0.008
Total rejection score	0.13 (0.00–0.25)	0.14 (0.00–0.40)	0.11 (0.00–0.19)	15.51 (2.15–84.90)	0.006
>12 mo after HTx	n = 73 (78%)	n = 21 (91%)	n = 52 (98%)		
No. EMBs	6 (3–11)	7 (5–12)	6 (3–11)		0.38[b]
Grade 1	36 (49%)	13 (62%)	23 (44%)	1.68 (0.69–4.07)	0.25
Grade 2	5 (7%)	1 (5%)	4 (8%)	0.59 (0.08–4.42)	0.61
Recurrent grade 1	15 (21%)	7 (33%)	8 (15%)	2.21 (0.89–5.51)	0.09
Total rejection score	0.00 (0.07–0.23)	0.11 (0.00–0.23)	0.00 (0.00–0.24)	4.52 (0.50–40.80)	0.18
>3 y after HTx	n = 56 (60%)	n = 17 (74%)	n = 39 (74%)		
No. EMBs	5 (3–9)	6 (4–10)	5 (3–9)		0.49[b]
Grade 1	19 (34%)	7 (41%)	12 (31%)	2.40 (0.77–7.41)	0.13
Grade 2	5 (9%)	1 (6%)	4 (10%)	0.67 (0.09–5.09)	0.70
Recurrent grade 1	7 (13%)	4 (24%)	3 (8%)	2.11 (0.67–6.50)	0.19
Total rejection score	0.00 (0.00–0.22)	0.00 (0.00–0.27)	0.00 (0.00–0.17)	3.21 (0.41–24.98)	0.27

Data are presented as n (%) or median (IQR), as appropriate.

[a]Effect of recipient and donor characteristics for CAV development analyzed by univariable Cox regression analysis unless otherwise stated.

[b]Comparisons between groups 1 and 2 by Mann-Whitney *U* test.

AMR, antibody-mediated rejection; CAV, cardiac allograft vasculopathy; CI, confidence interval; EMB, endomyocardial biopsy; HR, hazard ratio; HTx, heart transplant; IQR, interquartile range.

estimated incidences of CAV at 15 y were congruent.[2,12] Our complete follow-up in a unified setting without dropouts, an angiography protocol including only patient years or older, and an immunosuppression protocol may explain this modest difference. In our center, cyclosporine A–based immunosuppression has been used until relatively recently. However, we found no significant difference in CAV incidence between transplant eras. Notably, most patients with preexisting DSAs or immunoactivity in EMBs have been changed to tacrolimus and mycophenolate mofetil–based immunosuppression. All patients received triple immunosuppression; thus, our results do not support previous suggestions that triple immunosuppression may decrease incidence of CAV.[13,14]

Pretransplant DSAs were associated with CAV. A previous adult study with 10% incidence of pretransplant DSA also showed pretransplant DSA as a risk factor for CAV.[6] Furthermore, higher pretransplant calculated panel-reactive antibodies are a CAV risk factor based on ISHLT registry data.[15] However, evidence shows that patients with pretransplant DSAs may have less graft loss than patients with dnDSAs or without DSAs, presumably because of pretransplant antibody-depleting treatments.[16] In our study, all patients received polyclonal ATG induction therapy independently of pretransplant antibody status.

Posttransplant DSAs occurred in 43% of patients, of whom 92% had dnDSAs and 70% had HLA class II DSAs, corresponding to previous reports from pediatric HTx populations.[17,18] Adult studies have clearly revealed that dnDSAs, persistence of DSAs, and class II HLA DSAs are associated with adverse outcomes after HTx.[3,7,16,19] Also, pediatric patients with persistent DSAs, especially against class II HLA, have worse survival, whereas transient DSAs play a minor or no role in outcome.[17,18,20] DSAs play an integral role in the development of AMR, which in turn clearly associates with CAV.[3] In our study, posttransplant DSAs, neither persistent DSAs, DSAs against class II HLA, nor dnDSAs are associated with CAV risk. Although most of the AMR cases were in patients who developed CAV, we could not demonstrate an association between AMR and CAV with our limited sample size. Furthermore, a recent multicenter study on >2000 pediatric HTx patients showed no difference in graft loss between ABO-incompatible and ABO-compatible HTx.[21] In our data, ABO-incompatible HTx were few, preventing the reliable study of ABO-incompatibility and CAV risk.

Almost 70% of patients had at least 1 G1R, and 46% had recurrent G1R during follow-up. Typically, G1Rs are left untreated and not considered as rejection in retrospective studies. Thus, comparing G1R incidence between our

TABLE 4.

DSA findings before and after HTx among patients who developed CAV (group 1) and those who did not (group 2)

	All patients (N = 94)	Group 1, CAV (n = 23)	Group 2, no CAV (n = 53)	HR (95% CI)	P[a]
DSA findings before HTx	n = 78	n = 19	n = 48		
Any DSA	13 (17%)	7 (37%)	3 (6%)	3.78 (1.46–9.76)	0.006
HLA class					
Class I DSA	5 (38%)	2 (29%)	2 (67%)		
Class II DSA[b]	4 (31%)	2 (29%)	1 (33%)	1.43 (0.27–7.37)	0.69
Class I and II DSA	4 (31%)	3 (43%)	0 (0%)		
Cumulative MFI[c]	3267 (1174–24 370)	14 449 (1220–58 975)	1126 (1000–2114)[b]	1.00 (1.00–1.00)	0.63
DSA findings after HTx	n = 87 (93%)	n = 23 (100%)	n = 53 (100%)		
Any DSA	37 (43%)	12 (52%)	18 (34%)	1.81 (0.80–4.10)	0.16
Single DSA	15 (17%)	4 (17%)	10 (19%)		
Transient DSA	12 (14%)	3 (13%)	3 (6%)		
Persistent DSA[d]	10 (11%)	5 (22%)	5 (9%)	1.28 (0.47–3.47)	0.63
De novo DSA	34 (39%)	10 (44%)	17 (32%)	1.53 (0.67–3.49)	0.31
HLA class					
Class I DSA	11 (30%)	4 (17%)	6 (11%)		
Class II DSA[b]	12 (32%)	2 (9%)	7 (13%)	1.50 (0.64–3.54)	0.36
Class I and II DSA	14 (38%)	6 (26%)	5 (9%)		
DQ DSA	22 (25%)	7 (30%)	9 (17%)	1.68 (0.69–4.10)	0.25
No. DSA[c]	2 (1–3)	2 (1–4)	1.5 (1–3)	1.15 (0.98–1.34)	0.09
Highest cumulative DSA MFI[c]	7232 (4061–17 846)	8723 (4500–19 580)	5111 (2000–14 585)	1.00–1.00	0.28

Data are presented as n (%) or median (IQR), as appropriate.
[a]Effect of variables for CAV development analyzed by univariable Cox regression analysis.
[b]Patients with HLA class II DSA alone or with HLA class I DSA compared with the patients without HLA class II DSA.
[c]Patients with DSA included.
[d]Patients with persistent DSA compared with patients with single, transient, or no DSA.
CAV, cardiac allograft vasculopathy; CI, confidence interval; DSA, donor-specific antibody; EMB, endomyocardial biopsy; HR, hazard ratio; HTx, heart transplant; IQR, interquartile range; MFI, mean fluorescence intensity.

TABLE 5.

Multivariable models for cardiac allograft vasculopathy after heart transplant

	Model 1[a]			Model 2[b]		
	HR	95% CI	P	HR	95% CI	P
Presence of pretransplant DSA	3.59	0.77–16.61	0.10	3.54	1.36–9.22	0.01
Donor BMI	1.19	1.02–1.38	0.03			
Recurrent G1R ≤12 mo after HTx	12.1	2.97–49.57	0.0005	3.19	1.23–8.30	0.017
No. DSA after HTx	0.89	0.69–1.14	0.35			

[a]Model 1 includes covariates with $P < 0.1$ in univariable analysis.
[b]Model 2 includes covariates with $P < 0.05$ in univariable analysis.
BMI, body mass index; CI, confidence interval; DSA, donor-specific antibody; G1R, grade 1 rejection; HR, hazard ratio; HTx, heart transplant.

study and others is challenging.[22] In our study, severe G3Rs did not occur, whereas G2Rs appeared mainly during the first 3 mo and only in 12% compared with other pediatric HTx data showing 35% of patients having G2R or higher rejections.[22] Incidence of G2R was also low in our study when compared with ISHLT reports showing treated rejections during the first year in 13% of patients from 2010 to 2018 and 24% of patients from 2005 to 2009.[2] The low incidence of G2R or higher rejections may reflect the effect of triple immunosuppression and active treatment of G1R in our institute.

First-year G1R findings and G2R findings during the first 3 mo after HTx were associated with CAV, consistent with a previous pediatric study showing an association between ≥2 rejection episodes during the first posttransplant year and CAV.[23] Also, in a 2019 ISHLT report, a rejection episode in the first year after HTx was associated with CAV.[2] Other pediatric studies have also shown that patients with moderate or severe rejection after the first year are at higher risk for developing CAV, especially if clinical signs of rejection are present.[5,22] Contrary to these findings, rejections after the first year showed no association with CAV in our data. Recurrent G1R findings during the first year and during total follow-up were associated with CAV. Furthermore, during the first year after HTx, TRS did associate with CAV, which further emphasizes the effect of recurrent rejections on CAV development. In the adult HTx population, 6-mo TRS but not 12-mo was associated with earlier onset of CAV.[10] The association between recurrent mild rejections and CAV is also supported by previous data from pediatric and adult HTx populations demonstrating that recurrent mild (1B) rejections associate with worse graft function and patient outcome.[4,24] In contrast, Asimacopoulos et al[22] suggested that rejection diagnosed only on a protocol biopsy without decreased graft function does not associate with CAV development. Notably, approximately one-third of patients had G1R in their protocol EMBs even

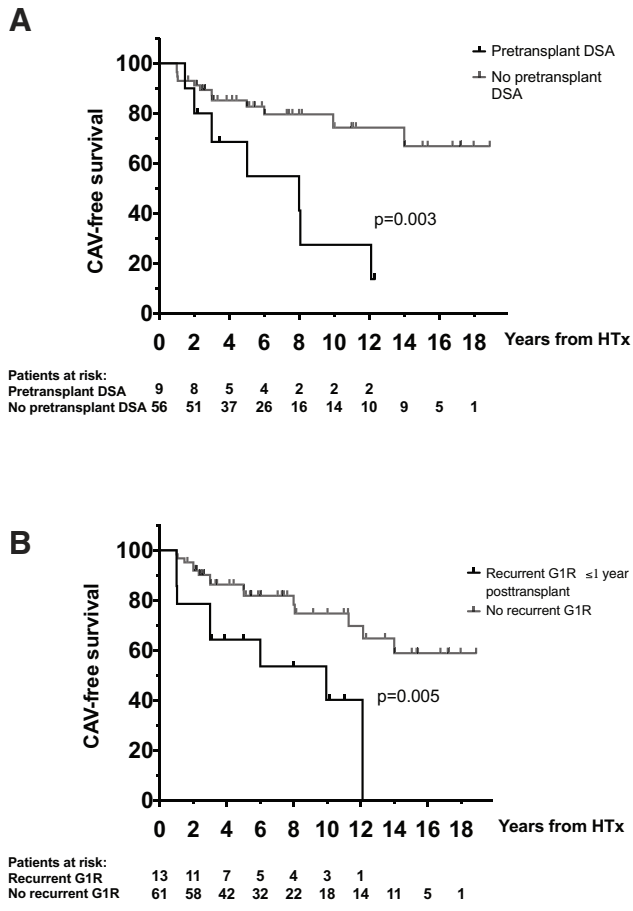

FIGURE 2. Log-rank comparison of Kaplan-Meier estimated CAV-free survival in patients with and without pretransplant DSA (A), and in patients with and without recurrent G1Rs during the first posttransplant year (B). CAV, cardiac allograft vasculopathy; DSA, donor-specific antibody; G1R, grade 1 rejection; HTx, heart transplant.

after 3 y post-HTx. Although no significant association with CAV risk was found, this may indicate poor compliance and warrant closer follow-up of these patients. This assumption is supported by the finding that great variability in immunosuppressive medicine levels associates with increased CAV incidence and poorer outcome after HTx.[25] Contrary to findings of pediatric registries, in our data, donor body mass index was the only recipient- or donor-related CAV risk factor linked to CAV.[2,12,15]

The present study has several limitations. First, the small sample size reduces the power to reliably analyze CAV risk factors; caution is needed especially when interpreting negative results. Second, the material stems from a single center, and follow-up ended at the transition to adult care. Third, CAV diagnoses were based on angiography findings, which may underestimate CAV prevalence.[26] Fourth, because this is a retrospective study, we could not correlate our findings with recently invented biomarkers, such as cell-free DNA or circulating microRNA, which could potentially replace EMB in rejection diagnostics.[27,28] Finally, dnDSA prevalence may be slightly underestimated because of incomplete typing for donor HLA-DP loci. However, our study population comprises a national cohort of pediatric HTx recipients with uniform treatment and comprehensive follow-up protocol without any dropouts, substantially strengthening our study results.

In conclusion, our data confirm previous findings that immune-mediated factors increase CAV risk after pediatric HTx, which is associated with poorer graft function and loss. Repetitive low-grade cell-mediated rejections, especially during the first posttransplant year, may not be as harmless as previously expected. However, these conclusions should be confirmed in larger patient populations.

ACKNOWLEDGMENTS

The authors thank Tero Vahlberg for the statistical assistance.

REFERENCES

1. Laks JA, Dipchand AI. Cardiac allograft vasculopathy: a review. *Pediatr Transplant.* 2022;26:e14218.
2. Rossano JW, Singh TP, Cherikh WS, et al; International Society for Heart and Lung Transplantation. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: twenty-second pediatric heart transplantation report—2019; focus theme: donor and recipient size match. *J Heart Lung Transplant.* 2019;38:1028–1041.
3. Barten MJ, Schulz U, Beiras-Fernandez A, et al. The clinical impact of donor-specific antibodies in heart transplantation. *Transplant Rev (Orlando).* 2018;32:207–217.
4. Feingold B, Irving C, Tatum GH, et al. Prognostic significance of recurrent grade 1B rejection in the first year after pediatric cardiac transplantation: a case for reinstatement of the 1B rejection grade. *Pediatr Transplant.* 2011;15:589–593.
5. Mulla NF, Johnston JK, Vander Dussen L, et al. Late rejection is a predictor of transplant coronary artery disease in children. *J Am Coll Cardiol.* 2001;37:243–250.
6. Frank R, Molina MR, Goldberg LR, et al. Circulating donor-specific anti-human leukocyte antigen antibodies and complement C4d deposition are associated with the development of cardiac allograft vasculopathy. *Am J Clin Pathol.* 2014;142:809–815.
7. Su JA, Baxter-Lowe LA, Kantor PF, et al. The clinical impact of donor-specific antibodies on antibody-mediated rejection and long-term prognosis after heart transplantation. *Curr Opin Organ Transplant.* 2019;24:245–251.
8. Raissadati A, Pihkala J, Jahnukainen T, et al. Late outcome after paediatric heart transplantation in Finland. *Interact Cardiovasc Thorac Surg.* 2016;23:18–25.
9. Stewart S, Winters GL, Fishbein MC, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant.* 2005;24:1710–1720.
10. Raichlin E, Edwards BS, Kremers WK, et al. Acute cellular rejection and the subsequent development of allograft vasculopathy after cardiac transplantation. *J Heart Lung Transplant.* 2009;28:320–327.
11. Mehra MR, Crespo-Leiro MG, Dipchand A, et al. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy—2010. *J Heart Lung Transplant.* 2010;29:717–727.
12. Kobayashi D, Du W, L'Ecuyer TJ. Predictors of cardiac allograft vasculopathy in pediatric heart transplant recipients. *Pediatr Transplant.* 2013;17:436–440.
13. Addonizio LJ, Hsu DT, Douglas JF, et al. Decreasing incidence of coronary disease in pediatric cardiac transplant recipients using increased immunosuppression. *Circulation.* 1993;88(5 Pt 2):II224–II229.
14. Braunlin EA, Hunter DW, Canter CE, et al. Coronary artery disease in pediatric cardiac transplant recipients receiving triple-drug immunosuppression. *Circulation.* 1991;84(5 Suppl):III303–III309.
15. Singh TP, Cherikh WS, Hsich E, et al; International Society for Heart and Lung Transplantation. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: twenty-fourth pediatric heart transplantation report—2021; focus on recipient characteristics. *J Heart Lung Transplant.* 2021;40:1050–1059.
16. Clerkin KJ, Farr MA, Restaino SW, et al. Donor-specific anti-HLA antibodies with antibody-mediated rejection and long-term outcomes following heart transplantation. *J Heart Lung Transplant.* 2017;36:540–545.

17. Irving CA, Carter V, Gennery AR, et al. Effect of persistent versus transient donor-specific HLA antibodies on graft outcomes in pediatric cardiac transplantation. *J Heart Lung Transplant.* 2015;34:1310–1317.
18. Tran A, Fixler D, Huang R, et al. Donor-specific HLA alloantibodies: impact on cardiac allograft vasculopathy, rejection, and survival after pediatric heart transplantation. *J Heart Lung Transplant.* 2016;35:87–91.
19. Moayedi Y, Fan CS, Tinckam KJ, et al. De novo donor-specific HLA antibodies in heart transplantation: do transient de novo DSA confer the same risk as persistent de novo DSA? *Clin Transplant.* 2018;32:e13416.
20. Dipchand AI, Webber S, Mason K, et al; CTOTC-04 Investigators. Incidence, characterization, and impact of newly detected donor-specific anti-HLA antibody in the first year after pediatric heart transplantation: a report from the CTOTC-04 study. *Am J Transplant.* 2018;18:2163–2174.
21. Urschel S, Ballweg JA, Cantor RS, et al. Clinical outcomes of children receiving ABO-incompatible versus ABO-compatible heart transplantation: a multicentre cohort study. *Lancet Child Adolesc Health.* 2021;5:341–349.
22. Asimacopoulos EP, Garbern JC, Gauvreau K, et al. Association of clinical rejection versus rejection on protocol biopsy with cardiac allograft vasculopathy in pediatric heart transplant recipients. *Transplantation.* 2020;104:e31–e37.
23. Pahl E, Naftel DC, Kuhn MA, et al; Pediatric Heart Transplant Study. The impact and outcome of transplant coronary artery disease in a pediatric population: a 9-year multi-institutional study. *J Heart Lung Transplant.* 2005;24:645–651.
24. Anguita M, Lopez-Rubio F, Arizon JM, et al. Repetitive nontreated episodes of grade 1B or 2 acute rejection impair long-term cardiac graft function. *J Heart Lung Transplant.* 1995;14:452–460.
25. Sirota M, Heyrend C, Ou Z, et al. Impact of tacrolimus variability on pediatric heart transplant outcomes. *Pediatr Transplant.* 2021;25:e14043.
26. Seipelt IM, Pahl E, Seipelt RG, et al. Neointimal inflammation and adventitial angiogenesis correlate with severity of cardiac allograft vasculopathy in pediatric recipients. *J Heart Lung Transplant.* 2005;24:1039–1045.
27. Scott JP, Ragalie WS, Stamm KD, et al. Total cell-free DNA predicts death and infection following pediatric and adult heart transplantation. *Ann Thorac Surg.* 2021;112:1282–1289.
28. Shah P, Agbor-Enoh S, Bagchi P, et al; GRAfT Investigators. Circulating microRNAs in cellular and antibody-mediated heart transplant rejection. *J Heart Lung Transplant.* 2022;41:1401–1413.