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ORIGINAL ARTICLE

Cardiac allograft vasculopathy in pediatric heart transplant recipients

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Summary

Metabolic parameters for coronary allograft vasculopathy (CAV) have not been well defined in children. CAV (by angiography or autopsy) was studied in 337 heart recipients on a cyclosporine-based steroid-sparing regimen. Freedom from CAV for all was 79% at 10 years. Fifty-nine patients (18%) developed CAV at a mean of 6.5 ± 3 years post-transplant. First year rejections were significantly higher in CAV, mean 2.3 vs. 1.4, $P = 0.003$, odds ratio (OR) 1.8. Rejection with hemodynamic compromise beyond 1 year post-transplant was associated with CAV, $P < 0.001$, OR 8.4. There was no significant correlation among human leukocyte antigen DR (HLA DR) mismatch, pacemaker use or homocysteine levels and the development of CAV. Maximum cholesterol and low density lipoprotein (LDL) levels were not significantly different. Neither diabetes nor hypertension was significant predictors of CAV on multivariate logistic regression analysis. In conclusion, frequent and severe rejection episodes may predict pediatric CAV. Neither glucose intolerance nor lipid abnormalities appeared to alter risk for CAV in this population.

Introduction

Graft arteriosclerosis can be a formidable challenge to the longevity of a transplanted organ [1]. In pediatric heart transplantation, post-transplant coronary artery disease is the hallmark of chronic rejection and a common cause of late mortality[2,3]. A relation between hyperlipidemia and coronary allograft vasculopathy (CAV) has been pursued for years [4]. More recently, evidence for an association between impaired glucose tolerance and CAV has been suggested [5]. The potential association of CAV with homocysteine levels has been explored [6,7]. A multitude of tissue, organ, and body factors may be operative in CAV, and many have been retrospectively studied [8–14]. Moreover, immunosuppressive agents are among a host of transplant-specific variables, which may accelerate coronary artery disease in the cardiac graft [15,16]. In children, a robust vascular tree and an anabolic growth factor milieu may protect against CAV in the majority of patients. However, the risks of re-transplantation may be confounded in children as the perennial hope of achieving long-term hemodynamic stability with a single heart transplant procedure fails to materialize in practice. The rapid expansion of a broad array of immunosuppressants with more powerful antiproliferative capabilities holds promise for more favorable long-term outcomes [17]. This study is a retrospective analysis of transplant and metabolic characteristics of pediatric heart transplant recipients with CAV.

Materials and methods

Patient population and immunosuppression

A total of 412 infants, children, and adolescents have undergone heart transplantation at Loma Linda University Children's Hospital between November 1985 and September 2004. Only patients who survived more than 1 year after transplant ($n = 337$) were included in this

retrospective analysis. Standard immunosuppressive protocol is steroid-free and consists of antithymocyte prophylaxis, cyclosporine, and azathioprine. Rejection is initially treated with bolus glucocorticoids. Ongoing rejection may require conversion to tacrolimus, mycophenolate and/or sirolimus. The main reasons for conversion to tacrolimus or sirolimus are recalcitrant rejection or renal insufficiency, respectively.

Analysis included all patients who developed CAV beyond the first year following the transplant, and did not have known pre-existing coronary artery disease. CAV was defined as the presence of over 50% luminal reduction in any coronary artery branch on selective coronary angiography or autopsy (ISHLT grade 3 or more). Coronary angiography was performed using size-appropriate Judkins catheters. Serial cine films were reviewed by one or more interventional pediatric cardiologist, and positive results were re-reviewed by the same cardiac team. Diagnosis was made by autopsy in 20 cases.

The following information was obtained on all study patients: age at transplant, gender, ethnicity, initial diagnosis (complex congenital heart disease, cardiomyopathy, and other), cold ischemia time, panel of reactive antibodies, development of CAV, time period between transplant and CAV, first-year rejections, late rejection alone, or with hemodynamic compromise, required use of a pacemaker, history of immunosuppressive agents prior to the development of CAV, development of hypertension, or post-transplant diabetes prior to CAV, donor's age, race, cytomegalovirus (CMV) status, HLA DR donor and recipient status, adenoviral detection by polymerase chain reaction (PCR) in recipient's blood or graft, and recipient's body mass index (BMI, calculated by dividing the weight in kilograms by the square of the height in meters) as a measure of excess weight. Results of the following laboratory tests carried out routinely per institutional clinical care protocol were documented: fasting cholesterol, LDL, HDL, hemoglobin A1C (HbA1C) as a measure of glycemic control over the preceding 3 months, fasting and/or random plasma glucose, and in 207 patients, adenoviral PCR.

Statistical analysis

Data were analyzed using SPSS version 10.0 for Windows. Standard descriptive and nonparametric statistics were used for demographic and continuous variables. Kaplan–Meier and Cox proportional hazard analyses were employed.

Results

Freedom from CAV for all was 79% at 10 years (Fig. 1). Actuarial freedom from CAV at 9 years (longest tacrolimus follow-up period) was 82.7% in the pretacrolimus era vs. 82.2% in those transplanted post-tacrolimus (after April 1995), $P = 0.98$. (Both eras were steroid-sparing with cyclosporine as initial immunosuppressant). Median age at transplant was 0.24 years (range 0–17.7 years). Ethnic breakdown (Table 1) was 56% Caucasian, 31% Hispanic, 7% African American, and 5% Asian. Fifty-nine (18%) had CAV at a mean of 6.5 ± 3 years post-transplant. There

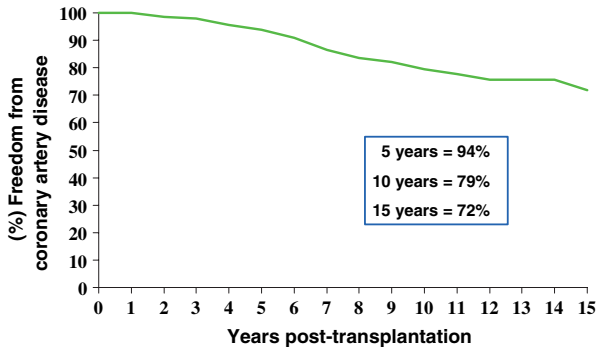

Figure 1 Freedom from coronary artery disease ($n = 59$).

Table 1. Characteristics of the study population for subjects with (cases) and without (controls) post-transplant coronary artery disease (CAV).

Characteristic	Noncases percents ($n = 278$)	Cases percents ($n = 59$)	*P*-value
Gender			
Males	39.9	42.4	0.77
Females	60.1	57.6	
Gender Mismatch			
No	49.3	55.9	0.39
Yes	50.7	44.1	
Race			
Black	5.8	11.9	0.53
Pacific/Asian	5.4	3.4	
Caucasian	56.5	54.2	
Hispanic	30.9	28.8	
Various	1.4	1.7	
Race mismatch			
No	39.9	55.9	0.03*
Yes	60.1	44.1	
Recipient Pre-Tx CMV			
Negative	68.3	55.9	0.07
Positive	31.7	44.1	
Donor CMV			
Negative	57.2	67.8	0.15
Positive	42.8	32.3	
HLA DR mismatch			
0	3.61%	5.17%	0.134
1	33.94%	46.55%	
2	62.45%	48.28%	

Analysis performed by chi-square. *$P < 0.05$.

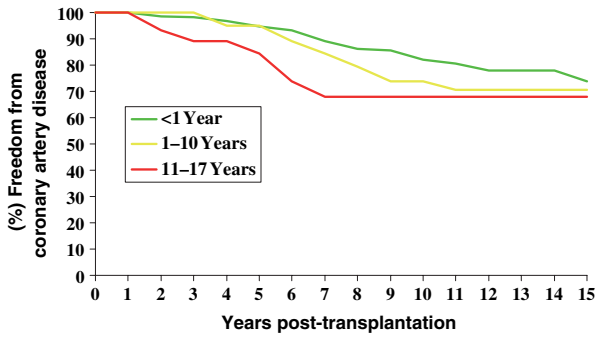

Figure 2 Age group analysis of post-transplant coronary artery disease (CAV).

Table 2. Characteristics of the study population for subjects with (cases) and without (controls) CAV.

Variable	Control $n = 278$ percent	CAV $n = 59$ percent	*P*-value
Statins			
No	75.9	57.6	0.01*
Yes	24.1	42.4	
Diabetes			
No	97.5	96.6	0.66
Yes	2.5	3.4	
Permanent pacemaker			
No	97.1	94.9	0.42
Yes	2.9	5.1	
Rapamycin			
No	75.5	76.3	0.99
Yes	24.5	23.7	
FK506			
No	83.5	93.2	0.07
Yes	16.5	6.8	
Methotrexate			
No	75.5	50.8	<0.001*
Yes	24.5	49.2	
Azathioprine			
No	15.8	18.6	0.57
Yes	84.2	81.4	
Mycophenolic acid			
No	70.9	64.4	0.35
Yes	29.1	35.6	
Total lymphocyte irradiation			
No	98.2	93.2	0.05
Yes	1.8	6.8	
Antithymocyte globulin prophylaxis			
No	38.1	45.8	0.31
Yes	61.9	54.2	
Antihypertensives			
No	71.2	64.4	0.35
Yes	28.8	35.6	

Analysis performed by chi-square. *$P < 0.05$.

were no significant differences in gender, BMI, or initial diagnosis. Analysis of cases by age group (Fig. 2) revealed a significant advantage to younger age. Donor/recipient race

Table 3. Selected characteristics for subjects with (cases) and without (controls) CAV.

Characteristic	Noncases mean rank ($n = 278$)	Cases mean rank ($n = 59$)	*P*-value
Survival	168.98	169.19	0.99
Age at Tx	167.15	177.73	0.45
Body mass index	152.67	164.42	0.40
Max Pre-Tx PRA	148.12	147.45	0.94
Cold ischemia time	171.02	159.49	0.41
Donor age	165.17	184.46	0.17
1st year rejection cnt	162.63	199.01	0.007*
Late rejection cnt	155.18	234.10	<0.001*
Late rejection rate	155.53	232.46	<0.001*
Hemo comp rejection cnt	155.14	234.32	<0.001*
Hemo comp rejection rate	155.46	232.80	<0.001*
Freedom from CAV	178.80	122.81	<0.001*
Adenovirus PCR	168.82	169.83	0.94

*$P < 0.05$ (Mann–Whitney *U*-test); Tx, transplant; PRA, panel reactive antibodies; Cnt, count; Hem comp, hemodynamic compromise.

mismatch was significantly lower in CAV ($P = 0.03$). Caucasian recipients of Hispanic donors had the least CAV ($P = 0.004$). An odd ratio (OR) for CAV was 21 for Asians ($P = 0.002$), and four for African Americans ($P = 0.08$). The HLA DR mismatch for one or both alleles was not a distinguishing factor ($P > 0.1$). Donor CMV status was negative in 68% of CAV vs. 56% of others ($P = 0.07$). Adenoviral PCR was not significantly prevalent in the CAV group. There was no significant correlation between pacemaker use and the development of CAV, $P > 0.4$ (Table 2). Methotrexate use but not antithymocyte prophylaxis or total lymphoid irradiation was significantly higher in CAV. Panel reactive antibodies and cold ischemia times were not significantly different (Table 3). First year rejections were significantly higher in CAV, mean 2.3 vs. 1.4, $P = 0.003$, OR 1.8. Rejection with hemodynamic compromise beyond 1 year post-transplant was associated with CAV, $P < 0.001$, OR 8.4. Maximum cholesterol and LDL levels were not significantly different. Twenty-two percent of patients (two of nine) with post-transplant diabetes developed CAV. Mean and maximum fasting glucose were 105 vs. 90 and 109 vs. 93 mg/dl ($P = 0.3$). Range of HbA1C for all was 4.5–12.5%. Maximum HbA1C was not significantly different for CAV patients (regardless of tacrolimus therapy). Neither diabetes nor hypertension appeared as significant predictors of CAV on multivariate logistic regression (Table 4). First year rejections and use of statin therapy appeared highly predictive of CAV on multivariate logistic regression. The actual year of transplant (1984–2004) was not a risk factor.

Table 4. Multivariable Cox proportional hazards regression for 13 study variables with CAV as the dependent variable and survival as the time variable (cases = 47) (subjects = 307).

Variable	Regression Coef. (β)	SE(β)	Increment (*k*)	Hazard ratio†	95% CI
Rej/HC	1.03274	0.35631	1	2.81*	1.40–5.65
FK	−0.92317	0.54488	1	0.40	0.14–1.16
Tx age (days)	−0.0000759	0.0002027	365	0.97	0.84–1.12
Gender	−0.24016	0.32537	1	0.79	0.42–1.49
Body mass index (BMI)	0.04816	0.06719	1	1.05	0.92–1.20
1st year rej	0.14398	0.08829	1	1.16	0.97–1.37
PTDM	−0.55465	0.85681	1	0.57	0.11–3.08
Donor CMV	−0.28358	0.34558	1	0.75	0.38–1.48
Race mis	−0.62601	0.31659	1	0.54*	0.29–0.99
Donor age (days)	0.0000633	0.0001332	365	1.02	0.93–1.13
Statins	0.43540	0.33513	1	1.55	0.80–2.98
Pre-Tx CMV	0.29999	0.33746	1	1.35	0.70–2.62
Anti-HBP	0.64501	0.33165	1	1.91	0.99–3.65

*$P < 0.05$.

†RR = exp(k*β).

Significance, $P < 0.05$; rej/hc, late rejection with hemodynamic compromise; FK, tacrolimus; Tx, transplant; rej, rejection, PTDM, post-transplant diabetes; mis, mismatch; anti-HBP, antihypertensives.

Discussion

In contradistinction to cardiovascular disease in the general population, CAV (also referred to as cardiac allograft vasculopathy) is characteristically more rapid, silent, and rarely associated with calcification [18]. As the hallmark of chronic rejection and a leading cause of mortality in pediatric and adult heart transplantation [19,20], efforts toward understanding its pathogenesis and prevention are pivotal to improved morbidity and mortality in this population.

Mechanisms for cardiac allograft vasculopathy have been divided into three, often inter-dependent categories [21]. The first presumed mechanism is immunologic, and involves acute rejection and anti-HLA antibodies. The second mechanism for the development of CAV is metabolic, relating to co-existent hypertension and/or diabetes in the donor or recipient. The third, and perhaps the most modifiable mechanism, relates to local and systemic effects of immunosuppressive agents. The detailed pathogenesis of CAV, however, remains unknown, though subclinical graft endothelial cell injury, because of ischemia-reperfusion damage or host versus graft attack, is the suspected-initiating insult.

Previous reports on CAV in pediatric heart transplant recipients [22] suggested that late rejection and donor/recipient racial matching were predictors of transplant vasculopathy. Though absolute frequency of CAV rose from 11% then to 17% in the current study, actual freedom from CAV at 10 years increased from 75% to 79%. This may reflect a lower frequency of rejection, a lower threshold for commencement of statin therapy or a more vascular sparing effect of newer immunosuppressive drugs. Both reports from our institution contrast with higher frequencies of CAV in the adult population, particularly those receiving maintenance steroid therapy [20].

The present study concurs with many reports linking frequent rejection episodes to CAV. Diagnosis at autopsy might have mis-represented the true prevalence of CAV. The role of tacrolimus, however, is not adequately addressed in view of its use in only a minority of study cases. Moreover, this study does not provide for discrepancies in the duration of tacrolimus therapy, thus, a dose-related effect cannot be established. *In vitro* data have also been perplexing. A CAV-protective effect of tacrolimus despite reported diabetogenic and nephrotoxic effects of the drug [23] has recently been suggested by studies of immunosuppressant effects on endothelial function. Tacrolimus, in therapeutic concentrations, uniquely did not induce oxidative stress or enhance nitric oxide production in human microvascular endothelial cells [24]. The interplay of tacrolimus in glucose homeostasis and induction or protection of CAV remains to be elucidated. Toxicity of tacrolimus to islets *in vitro* is in part related to a decrease in glucokinase activity, a key enzyme in glucose-induced insulin production [25]. Thus, resultant post-transplant diabetes is pathogenetically different from conventional types 1 and 2 diabetes with their notorious vascular complications. Moreover, as pointed out previously, CAV itself is etiologically and histologically different from cardiovascular disease in the nontransplant population. The literature thus far points to the need for a different preventive

approach for CAV, which will more than likely involve a step-wise risk-based individualized choice of immunosuppressants.

The negative correlation between donor CMV and CAV in current and previous studies [26] might imply the need for CMV prophylaxis in recipients at risk based on the data incorporating CMV in the induction of endothelial dysfunction[27]. Neither CMV nor adenoviral PCR data, however, were significant in our logistic regression model suggesting a possible surrogate effect. Protection against CAV in racially mismatched grafts may be related to genetic/racial variability in atherosclerosis inducers/markers such as TNF, TGF, CD25, CD30, CD69 (activation inducer molecule), CDw70, and CD16 [28]. It is unclear why Asian race was disproportionately more represented in CAV patients, a phenomenon which may be related to diet, or genetic variability in inflammatory markers or drug metabolism. Our data are somewhat limited by the lack of testing for specific or generalized markers of inflammation. Lack of an association between HLA DR mismatch and CAV confirms suggestions by earlier reports [29] that rejection, rather than mismatch, is the primary independent determinant of cardiac allograft outcome.

Another limitation of this study is the context of a large international program in which long-term follow-up data may not be available on all patients beyond 1 year of transplantation. Despite the vigilance of the transplant team in maintaining accurate follow-up data on all patients by telephone contact and annual record updates, coronary artery disease may be missed especially if asymptomatic. Long-term follow-up regarding compliance with the immunosuppressive regimen may similarly be incomplete, compromising the accuracy of the above results. Moreover, the frequency of CAV in this study was based on the total number of transplanted patients surviving their first year without consideration for the timing of CAV in relation to each of the immunosuppressive agents used. However, the total number of 1-year pediatric heart transplant survivors through September 2004 was 412, which would not dramatically affect the overall frequency reported.

The availability of information on CAV in heart transplant pediatric recipients from multiple centers, and the development of standardized protocols to define, diagnose, and treat CAV might elucidate further immunosuppressive links and facilitate preventive efforts.

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