A Pilot Study of Biomarkers for the Detection of Coronary Allograft Vasculopathy After Pediatric Heart Transplant

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ABSTRACT

Background: Cardiac transplantation is the definitive treatment for end-stage heart failure (ESHF), and over 350 pediatric orthotopic heart transplants (OHT) are performed yearly. In the pediatric population, this is the treatment option for uncorrectable congenital malformations, failed palliative procedures, cardiomyopathies, and ESHF. In the first year, survival is limited by acute rejection, infection, nonspecific graft failure, and multiorgan failure[2]. Beyond the first year, a major cause of mortality (affecting 50% of patients) is coronary allograft vasculopathy (CAV). Diagnosis remains difficult due to nonspecific symptomatology. We hypothesized that elevated biomarker levels are associated with CAV and may yield an improved diagnostic test for CAV.

Methods: After IRB approval, all patients undergoing cardiac catheterization after heart transplant at a tertiary children's hospital were approached for enrollment whereby a sample of blood was collected prior to the procedure. All patients who were given a diagnosis of CAV were matched with patients without disease based on age of allograft, age of patient, and patient sex in that order of importance. Samples were tested for levels of: c-reactive protein (CRP), troponin I (TnI), intercellular adhesion molecule-1 (ICAM-1), serum amyloid A (SAA), and brain natriuretic peptide (BNP) levels. In addition, the hemodynamic measurements made during cardiac catheterization were recorded in all patients for comparison. Nonparametric statistics were completed with Stata 14 Software (College Station, TX; USA), comparing biomarker levels and hemodynamic variables in patients with and without CAV.

Results: A total of 150 patients were enrolled in a 1-year period of this ongoing study. The 14 patients (9.3%) diagnosed with CAV were matched based on patient characteristics (table 2). There was an 80% increase in SAA levels in patients with (4.00 ng/mL) and without (2.17 ng/mL) CAV (p = 0.03). A multivariate logistic regression model improved ROC area to 0.82 (table 4). There was an increase in right ventricular systolic pressures in patients with CAV (33 mmHg vs. 25 mmHg, p = 0.02) (table 5).

Conclusions: The objective of this pilot study was to study biomarkers in the evolution of CAV in efforts to develop a multivariate biomarker panel to assess risk. In this preliminary 1-year analysis, SAA has been identified as a candidate biomarker. In addition, elevated right ventricular systolic pressures are associated with disease. In the future, a multivariate model of relevant biomarkers and physiological parameters may yield a tool that can allow for early diagnosis and improve outcomes in pediatric patients after OHT. With continual development, improved detection techniques may guide medical therapies and retransplantation strategies in the future.

BACKGROUND

Over 350 pediatric orthotopic heart transplants (OHT) performed yearly:[1]

- Uncorrectable congenital malformations; Failed palliations; Cardiomyopathies; End-stage heart failure.

Coronary allograft vasculopathy (CAV) is a leading cause of morbidity and mortality:[2]

- 50% of patients at 5-15 year follow up demonstrate angiographic evidence of CAV.
- Survival 67% at 1 year, 44% at 2 years, 17% at five years.

Challenging to diagnose:

- Denervated heart limits symptomatology.
- Sudden and unexpected deaths.

Surveillance:

- Coronary angiography (table 1)[3]
  - Sensitivity: 70 – 86%
  - Specificity: 92 – 99%
  - Positive predictive value 81 – 89%
  - Negative predictive value 77 – 99%

Limitations of angiography[4]:

- Under-diagnoses and underestimates severity.
- Mortality / morbidity risk of invasive procedure.

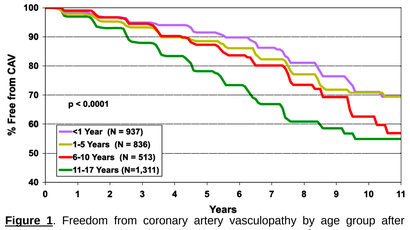

Figure 1. Freedom from coronary artery vasculopathy by age group after orthotopic heart transplantation (January 2000 - June 2013)[3].

Table 1. International Society for Heart and Lung Transplantation angiographic and functional nomenclature of CAV[3].

Grade	Description
CAV – 0 (not significant)	No detectable angiographic lesion
CAV – 1 (mild)	LM < 50%; any primary vessel < 70% or any branch <70%
CAV – 2 (moderate)	LM 50 – 70%; primary vessel ≥ 70%, or > in branches of 2 systems
CAV – 3 (severe)	LM ≥ 70%; 2 or more primary vessels ≥ 70%, or branch stenoses ≥ in all 3 systems
Functional upgrading	Evidence of significant systolic dysfunction (EF < 45%), restrictive hemodynamics, or CI < 2.1 L/min/m²

CAV = coronary allograft vasculopathy; LM = left main coronary artery; EF = ejection fraction; CI = cardiac index. If any functional abnormality is present in combination with CAV-1 or CAV-2 angiographic changes, the patient is upgraded to CAV-3.

The development of a noninvasive biomarker panel can help with diagnosis of coronary allograft vasculopathy after pediatric orthotopic heart transplant

METHODS

Study Design:

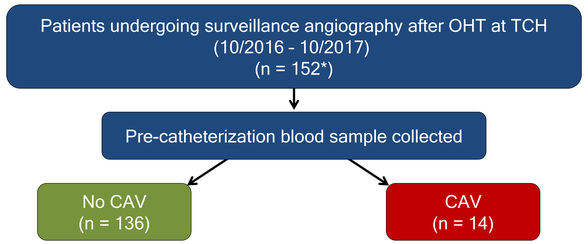

Figure 2. Flowchart of study design. OHT: orthotopic heart transplant. TCH: Texas Children's Hospital. CAV: coronary allograft vasculopathy. *Two patient families (1.3%) chose not to enroll in study.

Analysis:

- CAV matched controls (1:1) based on allograft age, patient age, sex (table 2).
- Samples tested for levels of c-reactive protein (CRP), troponin I (TnI), intercellular adhesion molecule-1 (ICAM-1), serum amyloid A (SAA), and brain natriuretic peptide (BNP).
- Hemodynamic measurements during cardiac catheterization recorded.
- Wilcoxon rank-sum test for continuous variables and Fisher's exact test for nominal variables.
- Multivariate logistic regression model of CAV created from biomarker levels.
- Calculations performed with Stata (StataCorp, College Station, TX) and statistical significance defined as p < 0.05.

RESULTS

Matched Controls:

Table 2. Patient population characteristics.

Characteristic	All (n = 28)	Control (n = 14)	CAV (n = 14)	p
Age (y)	13 [11 – 16.5]	13 [11 – 15]	13 [12 – 17]	0.55
Weight (kg)	53.5 [37.8 – 61.5]	51.7 [33.4 – 55.1]	55.8 [43.1 – 62]	0.44
Height (cm)	159.5 [146.5 – 164.3]	151.5 [133 – 169]	160.5 [148 – 164]	0.80
Allograft age (y)	9.71 [3.62 – 12.0]	9.93 [4.06 – 12.1]	9.55 [3.50 – 12.1]	0.78
Sex (M)	19 (67.9%)	10 (71.4%)	9 (64.3%)	1.00

Values are median [IQR_{25} – IQR_{75}] or number (percentage). CAV: coronary allograft vasculopathy. IQR: interquartile range.

Biomarker Analysis:

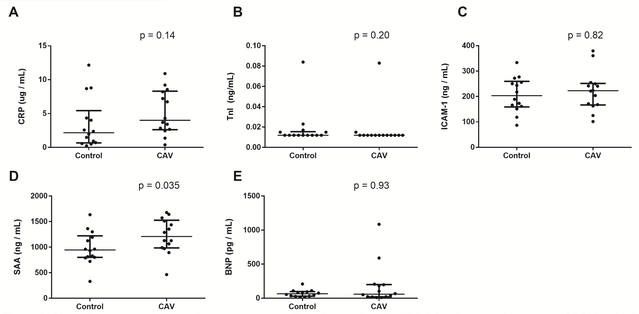

Figure 3. Biomarker analysis in control versus coronary allograft vasculopathy (CAV) for A: c-reactive protein (CRP) levels; B: troponin I (TnI) levels; C: intercellular adhesion molecule-1 (ICAM-1) levels; D: serum amyloid A (SAA) levels; E: BNP (brain natriuretic peptide) levels.

In coronary allograft vasculopathy, 27.9% increase in serum amyloid A levels (p < 0.05)

Table 3. Biomarker comparison between control and coronary allograft vasculopathy.

Biomarker	All (n = 28)	Control (n = 14)	CAV (n = 14)	p	ROC	95% CI
CRP (ug / mL)	3.13 [1.43 – 7.72]	2.17 [0.707 – 4.34]	4.00 [2.67-8.22]	0.14	0.66	[0.45 – 0.88]
TnI (ng / mL)	0.120 [0.120 – 0.135]	0.12 [0.12 – 0.15]	0.12 [0.12 – 0.12]	0.20	0.61	[0.23 – 0.56]
ICAM-1 (ng / mL)	220 [164 – 253]	204 [161 – 256]	222.7 [168.1 – 250.2]	0.82	0.53	[0.30 – 0.75]
SAA (ng / mL)	1095 [861.6 – 1358]	945.8 [803.0 – 1197]	1209.6 [990.3 – 1512.1]	0.035*	0.73	[0.54 – 0.93]
BNP (pg / mL)	61.6 [25.2 – 109]	65.4 [30.6 – 94.1]	59.9 [21.5 – 198.6]	0.93	0.51	[0.28 – 0.74]

Values are median [IQR_{25} – IQR_{75}]. CAV: coronary allograft vasculopathy. IQR: interquartile range. ROC: receiver operating characteristic (area under curve). CI: confidence interval. CRP: c-reactive protein. TnI: troponin I (TnI). ICAM-1: intercellular adhesion molecule-1. SAA: serum amyloid A. BNP: brain natriuretic peptide. *p < 0.05.

RESULTS

Logistic Regression Model:

Table 4. Logistic model of serum biomarker panel for diagnosis of coronary allograft vasculopathy.

Variable	Coefficient	p	ROC	95% CI
CRP (ug / mL)	0.044	0.75		[-0.22 – 0.31]
TnI (ng / mL)	0.043	0.16		[-533 – 86.8]
ICAM-1 (ng / mL)	0.0065	0.77		[-0.010 – 0.023]
SAA (ng / mL)	0.0030	0.093		[-0.0050 – 0.0065]
BNP (pg / mL)	0.016	0.12		[-0.0039 – 0.035]
Model			0.82	[0.65 – 0.98]

ROC: receiver operating characteristic (area under curve). CI: confidence interval. CRP: c-reactive protein. TnI: troponin I (TnI). ICAM-1: intercellular adhesion molecule-1. SAA: serum amyloid A. BNP: brain natriuretic peptide.

Hemodynamic Analysis:

Table 5. Hemodynamic comparison between control and coronary allograft vasculopathy.

Parameter	All (n = 28)	Control (n = 14)	CAV (n = 14)	p
RA Systolic (mmHg)	8 [7.5 - 10]	8 [6 - 10]	9 [8 - 12]	0.096‡
RA Diastolic (mmHg)	9 [7.5 - 10]	8 [7 - 10]	10 [8 - 11]	0.27
RA Mean (mmHg)	7 [5.5 - 8]	6 [5 - 7]	7 [6 - 9]	0.14
RV Systolic (mmHg)	27 [24 - 35]	25 [24 - 28]	33 [26 - 36]	0.022*
RV Diastolic (mmHg)	0 [0 - 2]	0 [0 - 0]	1.5 [0 - 3]	0.083‡
RV Mean (mmHg)	8 [8 - 10]	8 [7 - 9]	9.5 [8 - 11]	0.15
PA Systolic (mmHg)	25 [22 - 30]	24 [22 - 28]	26 [22 - 32]	0.16
PA Diastolic (mmHg)	11 [10 - 13]	10 [10 - 12.5]	11 [10 -14]	0.33
PA Mean (mmHg)	18 [16 - 20]	16.5 [15.5 - 19]	19 [16 - 24]	0.18
PCW (mmHg)	13 [10 - 16]	13 [11 - 14]	13 [10 - 16]	0.78
EF (%)	65 [61 - 66]	65 [61 - 68]	63.5 [59 - 66]	0.59
Diastolic Dysfunction	16 (57%)	7 (50%)	9 (64%)	0.70

Values are median [IQR_{25} – IQR_{75}]. CAV: coronary allograft vasculopathy. IQR: interquartile range. RA = right atrium. RV = right ventricle. PA = pulmonary artery. PCW = pulmonary capillary wedge. EF = ejection fraction. *p < 0.05. ‡p < 0.1.

In coronary allograft vasculopathy, combinations of biomarkers increase ROC area and disease is associated with 32% elevation in right ventricular systolic pressures (p < 0.05)

CONCLUSIONS

Coronary allograft vasculopathy:

- A high-risk condition affecting 50% of pediatric patients after heart transplant (5-15 years).
- Requires frequent invasive monitoring to prevent sudden cardiac arrest.

Biomarker analyses may yield improved detection of disease:

- Serum amyloid A, an apolipoprotein elevated in inflammatory disease, is associated with CAV[5].
- Combinations of biomarkers increase the diagnostic potential.

Future noninvasive methods of CAV detection:

- A biomarker panel is useful in diagnosis.
- Coupled with physiologic parameters, biomarker panels may have the greatest diagnostic potential.

Creation of a multivariate, noninvasive predictor of coronary allograft vasculopathy may lead to improved detection and could improve outcomes in this high-risk population

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