Feasibility of Real-Time Myocardial Contrast Echocardiography to Detect Cardiac Allograft Vasculopathy in Pediatric Heart Transplant Recipients

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Background: Cardiac allograft vasculopathy (CAV) is an important adverse prognostic factor for pediatric heart transplant (HT) recipients. Invasive coronary angiography (ICA) is the gold standard for CAV detection but lacks sensitivity for early microvascular changes and cumulative radiation exposure is of concern. Real-time myocardial contrast echocardiography (RTMCE) using ultrasound enhancing (contrast) agents performed during dobutamine stress echocardiography (DSE) can assess myocardial function, perfusion, and microvascular integrity. The objective of this study was to determine the safety and feasibility of RTMCE during DSE to detect CAV in a pediatric HT population.

Methods: HT patients 10-21 years of age were recruited to undergo DSE with RTMCE to determine technical feasibility, test tolerability and adverse event rate, and detection of perfusion defects compared with ICA-detected CAV. Thirty-six patients from two centers were enrolled, with a mean age 13.5 ± 4.3 years; 21 (58%) were male. Wall motion and myocardial perfusion were qualitatively assessed and compared with ICA findings of CAV. Myocardial blood flow (MBF) at rest and peak stress was quantified, and myocardial blood flow reserve (MBFR) was defined as the ratio of peak to rest MBF.

Results: Five (14%) patients had CAV by ICA, two with obstructive disease and three with mild CAV. Real-time myocardial contrast echocardiography was feasible in 32 (89%) patients. Three patients had wall motion defects, including one with a mixed defect and two with fixed defects. A perfusion abnormality was present in five patients, two of whom had obstructive CAV and one with mild CAV. Sensitivity and specificity of RTMCE for CAV detection were 60% and 94%, respectively, and diagnostic accuracy was 89%. MBFR assessment was feasible in 20 (63%) patients. The mean MBFR was 3.4 ± 0.7. Patients with CAV had lower MBFR than those without (2.0 ± 0.2 vs 3.7 ± 0.8; $P < .01$). There were no serious adverse events related to RTMCE.

Conclusions: Dobutamine stress RTMCE appears to be safe and feasible for the assessment of CAV in pediatric HT recipients. Further assessment is warranted to determine whether this noninvasive technique could provide a reliable alternative to ICA. (J Am Soc Echocardiogr 2021;34:503-10.)

Keywords: Real-time myocardial perfusion echocardiography, Ultrasound enhancing agent, Pediatric, Heart transplant, Cardiac allograft vasculopathy

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Abbreviations

CAV = Cardiac allograft vasculopathy

DSE = Dobutamine stress echocardiography

HT = Heart transplant

ICA = Invasive coronary angiography

IVUS = Intravascular ultrasound

LAD = Left anterior descending artery

LV = Left ventricular

MBF = Myocardial blood flow

MBFR = Myocardial blood flow reserve

MI = Mechanical index

rBV = Relative blood volume

RTMCE = Real-time myocardial contrast echocardiography

RWM = Regional wall motion

UEA = Ultrasound enhancing agent

Heart transplantation is a life-saving therapy for children with end-stage heart failure. Improved short-term graft survival has resulted in increased attention to managing long-term complications. Cardiac allograft vasculopathy (CAV) is a chronic graft complication whose prevalence increases over time[1] and is an important cause of morbidity and mortality among pediatric heart transplant recipients, accounting for approximately 25% of late deaths.[2,3] Cardiac allograft vasculopathy is a diffuse and accelerated intimal-proliferative process that affects both the large epicardial and small intramyocardial allograft coronary arteries. Early detection of CAV is critically important because it may allow alterations to medical therapy to slow progression before revascularization is required or possibly enable relisting for heart transplant or other interventions to prevent sudden death.[4] Early detection is challenged by the often asymptomatic nature of disease progression, making routine CAV screening the standard of care for heart transplant recipients. Angiographic imaging of the coronary arteries is the current reference standard for the detection of CAV, but it is invasive, often requires a general anesthetic in pediatric patients, exposes the patient to ionizing radiation, provides no information on graft function, and best evaluates the large vessels, with limitations for the detection of small-vessel disease. A noninvasive approach to serial CAV surveillance is therefore preferable; however, consensus regarding the best modality for this indication is lacking.

Dobutamine stress echocardiography (DSE) is a noninvasive stress imaging technique that has been advocated for routine CAV surveillance among adult heart transplant recipients[5] and studied in pediatric recipients.[6] More recently the sensitivity of this technique for CAV detection has been questioned.[7] Real-time myocardial contrast echocardiography (RTMCE) using ultrasound enhancing agents (UEAs) is a novel technique that allows simultaneous assessment of myocardial perfusion and wall motion during stress imaging.[8,9] Due to the characteristic diffuse microvascular involvement, RTMCE may represent a superior method for CAV detection because it identifies functional perfusion defects and provides an assessment of microvascular integrity.[10] However this approach has not been examined in a pediatric population. The purpose of this study was to assess the feasibility and safety of RTMCE for CAV detection in pediatric and young adult heart transplant recipients and compare findings with the reference standard invasive coronary angiography. We hypothesized that RTMCE would be well tolerated by pediatric heart transplant recipients without significant adverse events and that findings would correlate with the presence of CAV as detected by coronary angiography, suggesting that RTMCE may be an important noninvasive alternative approach to CAV screening in this population.

METHODS

This prospective feasibility study was conducted at two pediatric heart transplant centers, the Mayo Clinic (Rochester, MN) and the Alberta Children's Hospital (Calgary, AB, Canada). Heart transplant recipients between 10 and 21 years of age and >1 year post-transplantation were recruited from both centers at the time of clinically indicated invasive coronary angiography or within 6 months of invasive angiography to undergo RTMCE examination. For this feasibility study, recipients in this age range were chosen because they were considered to be more likely to tolerate the examination and also at relatively higher risk for CAV compared with younger recipients. The UEA used for this research protocol was perflutren lipid microspheres (Definity, Lantheus Medical Imaging, North Billerica, MA). Exclusion criteria included standard contraindications to receiving UEA including intracardiac shunt,[9] known hypersensitivity to perflutren, recent (<3 month) hospitalization for myocardial infarction/acute coronary syndrome, acute decompensated heart failure and/or acute allograft rejection, and multiorgan transplant. Five patients were excluded; four declined participation, and one was excluded due to a history of multiorgan transplant. Clinical, transplant, and medication data collected included etiology of pretransplant heart disease, recent laboratory testing including serum lipid levels, renal function and hemoglobin A1c, CAV risk factors (including hypertension, hyperlipidemia, diabetes mellitus, and renal disease), date of transplant, patient age at transplant, history of graft rejection, and current medications including immunosuppression therapy. Research ethics board approval was obtained from both institutions, and patient consent or assent and parental/guardian consent was obtained for all participants.

Dobutamine Stress Testing Protocol

Dobutamine stress testing was performed using a standardized protocol starting at a dose of 5 μg/kg/minute, followed by increasing doses of 10, 20, 30, to a maximal dose of 40 μg/kg/minute, in 3-minute stages, to achieve 75% of the age-predicted maximal target heart rate, calculated as 220 minus age in years. Atropine (maximum dose 2 mg) was used to achieve target heart rate if required once the maximal dobutamine dose rate was reached. Blood pressure and continuous cardiac rhythm were monitored before and during dobutamine infusion and during the recovery period after termination of the infusion until the heart rate returned to baseline. Patient monitoring during all examinations was supervised by an attending cardiologist.

RTMCE Imaging Protocol

Real-time myocardial contrast echocardiography examinations were performed as part of the research protocol for this study at the participating institutions using ultrasound machines (iE33, Philips Medical Systems, Andover, MA) equipped with broadband transducers and low-mechanical index (MI) contrast-specific presets. Apical four-, three-, and two-chamber views and short-axis views were acquired with low-MI 0.10-0.19, frame rates of 25-40 Hz, and a focus at the mitral valve level (for apical views), at baseline (prestress), low dose (dobutamine infusion rate 10 μg/kg/minute), and peak stress.[9,11] Time gain compensation and two-dimensional gain settings were adjusted and kept constant throughout the study, with potentiometers moved slightly higher in the near field.[9] A real-time destruction-replenishment technique was used in each view, with a transient (~10 frames) high-MI (1.0-1.3) imaging flash applied to destroy myocardial microbubbles, and subsequent replenishment

HIGHLIGHTS

- CAV causes significant morbidity in pediatric heart transplant recipients.
- CAV is difficult to detect by conventional noninvasive techniques.
- RTMCE during DSE was feasible and safe in 36 pediatric heart transplant recipients.
- Perfusion analysis identified CAV when compared with invasive coronary angiography.
- RTMCE may be a valuable approach to CAV screening in pediatric transplant patients.

was observed over a time period of 5-15 cardiac cycles. All images were stored digitally for offline analysis. The UEA solutions were prepared by drawing ≈0.75 mL (one half vial) into 30 mL syringes mixed with ≈29.25 mL normal saline for patients >60 kg, and for those ≤60 kg a dose of 20 μL/kg diluted to this same concentration was used. Slow hand bolus injections (simulating infusion) were administered through an intravenous line. The injection volume and rate were determined by visual monitoring of the left ventricular (LV) cavity and myocardial opacification, to keep a constant presence of microbubble effect without attenuation or shadowing. Injection with UEA was started 1 minute before RTMCE image acquisition at rest and was repeated at each dobutamine stage until completion of the imaging protocol.

Table 1 Baseline characteristics of the overall study population, and grouped according to presence or absence of angiographic CAV

Parameter	Study population (*N* = 36)	CAV (*n* = 5)	No CAV (*n* = 31)	*P* value
Clinical				
Age, years	13.5 ± 4.3	18.1 ± 1.6	12.9 ± 4.1	<.01*
Sex, male	21 (58)	2 (40)	19 (61)	.37
Body mass index, kg/m^2	20.2 ± 5.3	24.7 ± 5.1	21.5 ± 5.1	.09
Hypertension	5 (14)	1 (20)	4 (13)	.68
Diabetes mellitus	3 (8)	2 (40)	1 (3)	.02*
Hypercholesterolemia	5 (14)	1 (20)	4 (13)	.68
Renal insufficiency	9 (25)	1 (20)	8 (26)	.77
Transplant				
Age at transplant, years	6.7 ± 6.6	7.7 ± 6.1	6.2 ± 6.6	.26
Duration since transplant, years	7.1 ± 4.2	10.4 ± 4.7	6.6 ± 4.0	<.01*
Primary immunosuppression therapy				.38
Tacrolimus	13 (36)	2 (40)	11 (35)	
Sirolimus	18 (50)	3 (60)	15 (48)	
Cyclosporine	2 (6)	0	2 (6)	
Combination	3 (8)	0	3 (10)	
Additional immunosuppression therapy				.23
Mycophenolate mofetil	18 (50)	3 (60)	15 (48)	
Prednisone	5 (14)	1 (20)	4 (13)	
Azathioprine	3 (8)	0	3 (10)	
Medications				
Aspirin	7 (19)	2 (40)	5 (16)	.24
Statin	20 (56)	2 (40)	18 (58)	.64
Beta-blocker	4 (11)	1 (20)	3 (10)	.47
ACE inhibitor/ARB	7 (19)	0	7 (23)	.56
Laboratory testing				
eGFR, mL/minute/1.73 m^2	87 ± 27	70 ± 18	90 ± 28	<.01*
Hemoglobin, g/dL	13.1 ± 1.5	14.7 ± 2.3	12.9 ± 1.3	.15
White blood cell count, × 10^9/L	5.6 ± 2.1	5.4 ± 2.1	5.6 ± 2.1	.84
Platelet count, × 10^9/L	252 ± 68	213 ± 69	259 ± 67	<.01*
Total cholesterol, mg/dL	146 ± 29	161 ± 32	144 ± 28	<.01*
LDL cholesterol, mg/dL	74 ± 21	90 ± 25	71 ± 20	<.01*
HbA1c, %	5.5 ± 0.6	5.6 ± 0.5	5.5 ± 0.7	.98

Values in parentheses are percentages. *ACE*, Angiotensin converting enzyme; *ARB*, angiotensin II receptor blocker; *eGFR*, estimated glomerular filtration rate; *HbA1c*, hemoglobin A1c; *LDL*, low-density lipoprotein. *P* values compare values between patients with and without CAV.
*Statistically significant.

Table 2 Dobutamine stress testing characteristics of the overall study population, and grouped according to presence or absence of angiographic CAV

Parameter	Study population (*N* = 36)	CAV (*n* = 5)	No CAV (*n* = 31)	*P* value
Rest				
Heart rate, BPM	96 ± 13	94 ± 19	96 ± 12	.31
Systolic blood pressure, mm Hg	105 ± 17	117 ± 11	103 ± 17	.16
Diastolic blood pressure, mm Hg	61 ± 13	74 ± 14	59 ± 13	.01*
LV ejection fraction, %	63 ± 7	61 ± 7	63 ± 3	.27
Peak stress				
Heart rate, BPM	164 ± 14	161 ± 12	164 ± 15	.19
Systolic blood pressure, mm Hg	133 ± 30	142 ± 37	132 ± 29	.03*
Diastolic blood pressure, mm Hg	71 ± 21	85 ± 24	69 ± 21	<.01*
Heart rate × blood pressure product	21,269 ± 4,588	23,029 ± 7,960	21,049 ± 4,222	<.01*
LV ejection fraction, %	74 ± 6	70 ± 9	74 ± 5	.07

BPM, Beats per minute. *P* values compare values between patients with and without CAV.
*Statistically significant.

Image Interpretation

Qualitative Assessment of RWM and RTMCE. Interpretation of regional wall motion (RWM) and RTMCE imaging was performed by a single observer (R.H.) with over 5 years of analysis experience with this technique who was blinded to all clinical and angiographic data. The 17-segment LV model was used to evaluate myocardial wall motion and perfusion simultaneously.[12] Regional wall motion was evaluated using established American Society of Echocardiography grading criteria.[11] Resting and peak stress myocardial perfusion was scored for each stage and myocardial segment as either normal or abnormal based upon observed presence and timing of replenishment of microbubble enhancement within five cardiac cycles after flash depletion at rest or within two cardiac cycles at peak stress.[9] Complete replenishment and enhancement was scored as normal perfusion, while abnormal perfusion was scored if it was delayed or absent. Segments were scored as noninterpretable if not visualized or in the presence of attenuation. Attenuation was defined as decreased replenishment and overall enhancement within a segment that was present before and after the high-MI impulse associated with decreased signal intensity in adjacent nonmyocardial regions surrounding the segment of interest. Comparison of rest and stress RTMCE segmental scores was then performed for both RWM and perfusion; observed defects were further classified as reversible (observed during dobutamine infusion only and not observed under resting conditions), fixed (observed both at rest and stress without a change during dobutamine infusion), or mixed (combination of reversible and fixed defect). Any adverse effects observed within 1 hour of dobutamine infusion and UAE administration were recorded.

Quantitative Analysis. Biplane Simpson quantitation was used to calculate resting and peak stress LV ejection fraction.[11] Digitized RTMCE images were analyzed offline using dedicated software (QLAB version 10.0, Philips Medical Systems) by the same experienced and blinded observer (R.H.). Quantitative myocardial perfusion analysis was performed as described elsewhere.[10,13] Briefly, segmental regions of interest of standardized size and shape were placed on end-systolic frames starting in the frame immediately after the flash and were automatically copied to subsequent end-systolic frames that were manually adjusted to avoid overlapping cavity signals. The software automatically corrected for noncontrast signals arising from the tissue by subtracting the signal intensity of the first frame after bubble destruction. Time-intensity replenishment curves were generated and subsequently fitted to the following monoexponential function conventional equation: $y = A\ (1 - e^{-\beta t})$.[14] Absolute myocardial blood flow (MBF, mL/minute/g) was calculated using the model described by Vogel *et al.*[15] Additional regions of interest (of the same size as the myocardial regions of interest) were manually tracked in the adjacent LV cavity, and the LV intensity (A_{LV}) was obtained from averaging of the adjacent LV signal intensities for all end-systolic frames except the ones during the flash and the first frame after the flash. The relative blood volume (rBV; mL/mL) was calculated from the following formula: $rBV = A/A_{LV}$. The replenishment curve parameter, β (sec^{-1}), was then converted to β ($minute^{-1}$) to reflect the exchange flow velocity. Absolute MBF was calculated

Table 3 Noninvasive DSE (wall motion) and RTMCE findings of patients with angiographic CAV

Patient	ICA CAV Grade	IVUS	Rest wall motion	Stress wall motion	Rest perfusion	Stress perfusion
20-year-old male	2	Mild eccentric plaque	Abnormal	Abnormal	Abnormal	Abnormal
18-year-old female	1	Mild eccentric plaque	Normal	Normal	Normal	Normal
18-year-old female	1	Mild intimal thickening	Abnormal	Abnormal	Abnormal	Abnormal
16-year-old male	1	Not done	Normal	Normal	Normal	Normal
16-year-old female	2	Not done	Normal	Normal	Normal	Abnormal

ICA, Invasive coronary angiography.

Table 4 Noninvasive DSE (wall motion) and invasive (ICA and IVUS) findings in those patients with abnormal RTMCE

Patient	ICA CAV grade	IVUS	Rest wall motion	Stress wall motion	Rest perfusion	Stress perfusion
20-year-old male	2	Mild eccentric plaque	Abnormal	Abnormal, mixed defect	Abnormal	Abnormal
13-year-old male	0	Normal	Abnormal	Abnormal, fixed defect	Abnormal	Abnormal
11-year-old male	0	Not done	Normal	Normal	Normal	Abnormal
18-year-old female	1	Mild intimal thickening	Abnormal	Abnormal, fixed defect	Abnormal	Abnormal
16-year-old female	2	Not done	Normal	Normal	Normal	Abnormal

ICA, Invasive coronary angiography.

from the product of rBV $\times$ β divided by myocardial tissue density (1.05 g/mL). The MBF reserve (MBFR) values were calculated for each perfusion parameter as stress values divided by baseline values. Failure of curve fitting either at rest or stress resulted in excluding the segment from the analysis, and the segment was considered not analyzable. Curve fitting usually fails if the input image quality is poor, if the segment is not visualized entirely from the endocardial to the epicardial border, or if the intensity values do not model well with the monoexponential function equation. Overall feasibility of quantitative perfusion analysis was based on the available reserve values from all LV segments.

Invasive Coronary Angiography

Using clinically indicated invasive coronary angiography findings, CAV was graded (0-3, where 0 = no significant CAV, 1 = mild CAV, 2 = moderate CAV, and 3 = severe CAV) according to the International Society of Heart and Lung Transplantation criteria (see Supplemental Material for complete criteria) by an experienced operator (J.H.A.) blinded to all clinical and echocardiographic data.[16] Intravascular ultrasound (IVUS) of the left anterior descending artery (LAD) was performed as clinically indicated, and the presence or absence of CAV was determined by the same operator.[16] At the participating centers, routine surveillance invasive coronary angiography is typically performed every 1-2 years and/or as clinically indicated, and IVUS imaging is performed during angiography starting at age 10-12 years and/or as clinically indicated.

Statistical Analysis

Continuous data are reported as mean $\pm$ standard deviation and were compared using paired and unpaired *t* tests. Frequencies are used to report categorical variables, which were compared using χ^2 tests. Myocardial perfusion assessment sensitivity and specificity were performed using 2 $\times$ 2 contingency tables in comparison with the reference standard invasive coronary angiography. A true positive was defined as the presence of a fixed or reversible perfusion defect corresponding with CAV grade $\geq$1 by coronary angiography. Sample size calculations were not performed for this feasibility study. All statistical analysis was performed using commercially available software (Stata, ver. 14.2, StataCorp, College Station, TX). A two-sided *P* value $<$ .05 was considered statistically significant.

RESULTS

A total of 36 patients participated, and baseline clinical, transplant, medication, and laboratory testing data are provided in Table 1 for the study group and for those with and without angiographic CAV. Patients with CAV were significantly older, had a higher rate of diabetes mellitus, a longer duration since transplant, lower estimated glomerular filtration rate and platelet count, and higher total and low-density lipoprotein cholesterol levels. The indication for heart transplantation was complex congenital heart disease in 15 (42%) patients, dilated cardiomyopathy in 13 (35%), restrictive cardiomyopathy in 4 (11%), hypertrophic cardiomyopathy in 2 (6%), arrhythmogenic right ventricular cardiomyopathy in 1 (3%), and refractory arrhythmia secondary to congenital long QT syndrome in 1 (3%). Prior to heart transplantation, 10 (28%) patients were supported by a mechanical circulatory support device, including five (14%) with an LV assist device and five (14%) with extracorporeal membrane oxygenation. Ten (28%) patients had a prior history of biopsy-proven graft rejection, and none had active rejection at the time of study participation. No patients had a history of coronary artery revascularization.

Dobutamine Stress Testing

Characteristics of dobutamine stress testing are presented for the study group and for patients with and without angiographic CAV in Table 2. Patients with CAV had a higher resting diastolic blood pressure and a higher systolic and diastolic blood pressure and heart rate $\times$ blood pressure product at peak stress. The mean dose of dobutamine infusion at peak stress was 36 $\pm$ 7 μg/kg/minute, and 29 (81%) required the maximum dose of 40 μg/kg/minute, while 21 (58%) required administration of atropine (mean dose, 0.9 $\pm$ 0.5 mg; range, 0.25-2 mg). Thirty-three (92%) patients achieved the maximal age-predicted target heart rate (mean, 85% $\pm$ 16%). During dobutamine stress testing no patients developed ST-segment changes by continuous cardiac monitoring.

Dobutamine stress testing was well tolerated in most patients. Twelve (33%) experienced mild adverse side effects during testing. These included seven who experienced palpitations, among whom three also had headache and one had nausea, while three patients had headache alone and one had dyspnea. One (3%) patient required early termination of the test at 10 μg/kg/minute dobutamine dose due to severe headache. It is suspected that all adverse side effects were caused by dobutamine infusion rather than the UEA, as no patient experienced an adverse event after administration of UEA during rest imaging (prior to starting dobutamine); however, the true cause for these side effects cannot be determined. There were no serious adverse events. Variable levels of sedation were used for 16 (44%) patients who underwent DSE with RTMCE testing and only in the cardiac catheterization laboratory during the same visit as coronary angiogram, while 11 (31%) patients did not require sedation during DSE with RTMCE performed during coronary angiography. The remaining nine (25%) patients underwent DSE with RTMCE on a separate visit that was within 6 months of coronary angiography,

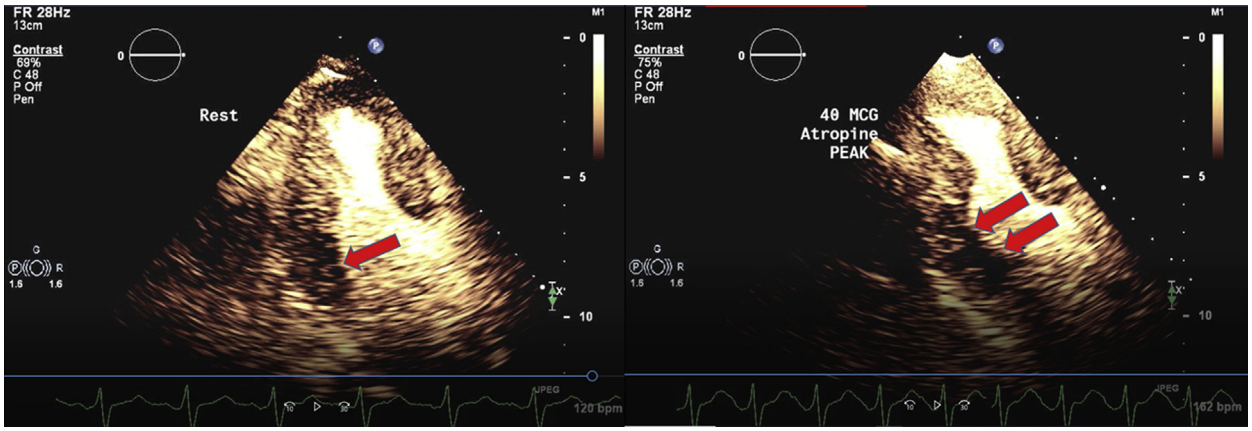

Figure 1 End-systolic apical three-chamber view still frames during RTMCE examination in a patient with grade 2 CAV, at rest (*left*) and during peak dobutamine stress (*right*). Resting perfusion is slightly delayed in the basal inferolateral wall (*arrow*), possibly due to basal attenuation. At peak stress, a well-demarcated subendocardial perfusion defect (*arrows*) is present (see Supplemental Video 1 for corresponding RWM assessment).

with a median time between DSE with RTMCE and coronary angiography of 4 months (range, 1-6 months).

Invasive Coronary Angiography

Five (14%) patients had angiographic CAV, including three (8%) with grade 1 CAV and two (6%) with grade 2 CAV (Table 3). Intravascular ultrasound imaging was performed on a total of 13 (36%) patients, with three having abnormal results (two of the patients with angiographic CAV did not undergo IVUS). The remainder of the patients had normal examinations.

RTMCE

Regional Wall Motion Assessment. Regional wall motion assessment was feasible in all 36 of patients included. Three (8%) patients had resting and/or peak stress RWM abnormalities (Table 4). These included one patient with a mixed defect during dobutamine stress who had grade 2 CAV by angiography and two patients with a fixed defect during dobutamine stress, one with grade 1 CAV by angiography and the other with grade 0 CAV.

Myocardial Perfusion Assessment. Myocardial perfusion assessment was feasible in 32 (89%) patients, while image quality was not adequate for perfusion assessment in four (11%) patients. Five (14%) patients demonstrated abnormal perfusion findings (Table 4). Two of these patients had angiographic grade 2 CAV, one patient had grade 1 CAV, and two patients had grade 0 CAV, including one with normal IVUS imaging and one with IVUS not performed. Both patients with grade 2 CAV had abnormal stress myocardial perfusion by RTMCE. One had >50% middle left circumflex artery stenosis with diffuse distal LAD narrowing by angiography, with abnormal rest and stress myocardial perfusion and abnormal rest and stress wall motion assessment (Figure 1, Supplemental Video 1, available at http://www.onlinejase.com/). The other patient with angiographic grade 2 CAV had a 70% proximal LAD stenosis and diffuse right coronary artery disease. This patient had normal rest but abnormal stress myocardial perfusion and, interestingly, had normal rest and stress wall motion. Of the two patients with abnormal RTMCE results but angiographic grade 0 CAV, one had abnormal rest and stress myocardial perfusion and wall motion and the other had normal rest but abnormal stress myocardial perfusion and normal rest and stress wall motion. Lastly, two patients had angiographic grade 1 CAV, but had both normal rest and stress myocardial perfusion and wall motion. Overall, these results are consistent with a sensitivity of 60%, a specificity of 94%, and a diagnostic accuracy of 89% for detecting angiographic CAV using RTMCE.

The quantification of MBFR was feasible in 20 (56% overall, 63% of patients with interpretable RTMCE) patients, including three with angiographic CAV. It was not feasible in the remainder due to patient factors including technically limited image quality owing to patient movement and/or high heart rates during the examination. Overall, the mean resting MBFR was 3.4 ± 0.7, and for patients with and without angiographic CAV, it was 2.0 ± 0.2 and 3.7 ± 0.8 respectively, $P < .01$.

DISCUSSION

Our study explored the safety and feasibility of dobutamine stress RTMCE using UEAs as a novel method to detect CAV in pediatric heart transplant recipients. This approach appears to be a safe method for assessment of global and regional LV function and perfusion during stress imaging without occurrence of significant adverse events. Myocardial perfusion assessment demonstrated modest sensitivity with higher specificity for the detection of angiographic CAV. Patients with angiographic CAV had significantly impaired microvascular function (as measured by MBFR), an important finding consistent with a disease known to significantly impact graft microvasculature. Of note, while qualitative myocardial perfusion assessment was feasible in most patients, MBFR was quantifiable in just over half owing to technical factors relating to image quality required for quantitative analysis. The RTMCE imaging adds minimal time to the performance of a standard DSE, with simultaneous qualitative interpretation of perfusion images with RWM assessment and an additional 5-10 minutes per examination for offline MBFR calculation. Further research is warranted to determine the diagnostic utility of RTMCE for routine CAV surveillance testing and whether the feasibility of MBFR quantification can be improved. In addition, this study adds to the growing body of evidence demonstrating the safety of UEAs in children, even in populations as medically complex as heart transplant recipients.

The diffuse, small-vessel nature of CAV presents a significant challenge for surveillance using conventional approaches. In light of this,

perfusion imaging represents a potentially valuable approach for this indication, and previous reports have examined the utility of techniques such as coronary flow reserve measurement using positron emission tomography[17] and Doppler echocardiography.[18] Our group has demonstrated RTMCE to be a feasible noninvasive method to assess myocardial microvascular function,[13] including in diabetic patients without significant epicardial coronary artery disease.[10] Real-time myocardial contrast echocardiography has also previously been examined for CAV detection in comparison with conventional DSE, nuclear scintigraphy perfusion imaging, and invasive coronary angiography with IVUS in adult heart transplant recipients.[19] That study reported incremental improvement in diagnostic accuracy over DSE from 80% to 85% with the addition of visual myocardial perfusion assessment and further increase to 90% by adding quantitative grading (similar to the MBFR calculation used in our study). Weis *et al.* [20] described endothelium-independent microvascular dysfunction and its prognostic importance for deterioration of LV function in heart transplantation recipients without angiographically visible coronary artery stenoses, supporting the concept that microvascular and epicardial vessel disease after transplantation may be distinct processes with different functional consequences.[19,20]

Importantly, in our study there was not complete agreement with the invasive coronary angiography findings in our cohort. There were two (6%) patients with no angiographic evidence of CAV (grade 0) who had abnormal RTMCE findings. There were also two patients with mild angiographic CAV (grade 1) who had normal RTMCE findings. This result is difficult to characterize and may reflect limitations of the technique. The small number of patients with CAV in our cohort limits our ability to draw definitive conclusions with respect to diagnostic accuracy and demonstrates the need for further investigation of RTMCE utility in this setting. Similarly, only a small number of patients (2, 6%) had normal resting but abnormal stress perfusion RTMCE imaging. The incremental value of stress imaging above rest perfusion RTMCE requires larger studies with a higher prevalence of CAV to determine.

Our study adds further data to the already published evidence supporting the safety of UEAs in pediatric echocardiography.[21-23] A recent retrospective single-center study describing clinical use of UEA in 115 children and young adult patients reported 13 patients experiencing adverse events or symptoms, all being transient and resolving without further sequelae or alteration of the imaging protocol.[24] That study included a wide range of different echocardiography indications for UEA, including both rest and stress imaging protocols in addition to RTMCE analysis. The authors concluded that UEAs are useful and safe in older pediatric patients in clinical practice. Our study contributes further to the growing body of literature now supporting the use of UEAs in children.

Limitations

Our study was designed to evaluate the safety and feasibility of a novel method for CAV detection in the pediatric heart transplant population; therefore sample size calculations to effectively determine the diagnostic accuracy of RTMCE were not performed. Our small sample size combined with few patients having angiographic CAV limits our ability to determine the diagnostic accuracy of RTMCE for this indication. The sensitivity of RTMCE for detection of angiographic CAV was only 60% in our study, suggesting that while our findings are hypothesis generating, this technique may also have limitations for this indication and additional studies are needed with a higher sample size and, importantly, a higher prevalence of CAV before wider clinical adoption could be considered. We did not administer UEA by continuous pump infusion, but rather by a slow hand injection. This has been shown to be more feasible and adequate for visual qualitative interpretation of myocardial perfusion, but ideally MBFR quantitative determinations should be done using an infusion pump to assure steady-state conditions. Not all patients underwent IVUS imaging in our study, the performance of which was guided by clinical judgment at the time of coronary angiography. As described above, invasive coronary angiography, even with the use of IVUS imaging, presents limitations as a reference standard for the detection of CAV due to the diffuse and often small-vessel nature of this process, which may have accounted for some of the discrepancies observed in our study between RTMCE and angiography. Furthermore, optical coherence tomography is another invasive technique demonstrating utility for detecting CAV in pediatric and adult heart transplant recipients,[25,26] but it was not performed for this study. Our study only included older children and young adult patients, and we cannot comment on the safety or feasibility of RTMCE for younger (<10 years of age) pediatric heart transplant recipients.

Conclusion

This prospective study found that RTMCE appears to be a safe and feasible method of CAV detection in children and young adult heart transplant recipients. Real-time myocardial contrast echocardiography was well tolerated with no significant adverse events. Myocardial perfusion assessment was available in most patients, and MBFR quantification was feasible in a smaller majority, although our study was underpowered to definitively determine diagnostic utility. Significant microvascular dysfunction as assessed by MBFR quantitative analysis was demonstrated in patients with angiographic CAV relative to those without CAV. Notably, while the diagnostic accuracy was 89%, the sensitivity of RTMCE to detect angiographic CAV was only 60%, limiting our ability at this time to recommend this technique as an approach to reducing the need for coronary angiography in this population. Further research in a larger cohort of patients is warranted to determine the clinical utility of RTMCE for routine CAV surveillance in younger heart transplant patients.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at https://doi.org/10.1016/j.echo.2020.12.009.

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