Myocardial Vascular Function Assessed by Dynamic Oxygenation-sensitive Cardiac Magnetic Resonance Imaging Long-term Following Cardiac Transplantation

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Background. Coronary vascular function is related to adverse outcomes following cardiac transplantation (CTx) in patients with or without cardiac allograft vasculopathy (CAV). The noninvasive assessment of the myocardial vascular response using oxygenation-sensitive cardiac magnetic resonance (OS-CMR has not been investigated in stable long-term CTx recipients). **Methods.** CTx patients were prospectively recruited to complete a CMR study with a breathing maneuver of hyperventilation followed by a voluntary apnea. Changes in OS-sensitive signal intensity reflecting the myocardial oxygenation response were monitored and expressed as % change in response to these breathing maneuvers. Myocardial injury was further investigated with T2-weighted imaging, native and postcontrast T1 measurements, extracellular volume measurements, and late gadolinium enhancement. **Results.** Forty-six CTx patients with (n=23) and without (n=23) CAV, along with 25 healthy controls (HC), were enrolled. The OS response was significantly attenuated in CTx compared with HC at the 30-second time-point into the breath-hold (2.63%±4.16% versus 6.40%±5.96%; $P=0.010$). Compared with HC, OS response was lower in CTx without CAV (2.62%±4.60%; $P<0.05$), while this response was further attenuated in patients with severe CAV (grades 2–3, –2.24%±3.65%). An inverse correlation was observed between OS-CMR, ventricular volumes, and diffuse fibrosis measured by extracellular volume mapping. **Conclusions.** In heart transplant patients, myocardial oxygenation is impaired even in the absence of CAV suggesting microvascular dysfunction. These abnormalities can be identified by oxygenation-sensitive CMR using simple breathing maneuvers.

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INTRODUCTION

Cardiac allograft vasculopathy (CAV) is one of the leading causes of long-term mortality following cardiac transplantation (CTx).[1] This condition is characterized by diffuse and concentric thickening of epicardial and intramyocardial graft vessels[2] and is associated with endothelial lesions, smooth muscle cell proliferation, and proinflammatory circulating cell accumulation in the intima, leading

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N.I. contributed to the design of the study, recruited the patients, interpreted the results, and wrote the article. K.F. provided significant contributions to the design of the study, data interpretation, cowrote the article, and had a significant role in the development of the OS-technique applied to cardiac patients. T.H. played a significant role in the software development and in the interpretation of the data. He reviewed the article and provided significant feedback to improve the content. M.F. contributed to the design of the study, had a significant role in the development of the OS-technique applied to cardiac patients, and provided the software needed to interpret this data. F.-P.M. contributed to the data analysis and interpretations and provided in depth review of the article to improve the intellectual content. M.W. cosupervised the graduate student (N.I.) with M.F., contributed to recruiting the patients, provided multiple reviews of the article to improve the intellectual content, and provided the funding for the realization of the study.

M.G.F. is a board member, advisor, and shareholder of Cir Cardiovascular Imaging, Inc., the manufacturer of the software used for CMR image evaluation. M.G.F. and K.F. were inventors of but no longer held the international patent: "Measuring oxygenation changes in tissue as a marker for vascular function." Initial Filing Date: August 8, 2013. Patent issued: April 11, 2017. Application number: 14/419,877. Patent number: 9615754. Continuation filing date: April 10, 2017. United States patent application no. 15/483,712, patent pending. As of April 2018, the patent rights were transferred to Cir Cardiovascular Imaging Inc., Calgary, AB, Canada.

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to myocardial injury without apparent epicardial coronary artery disease.[3] Microvascular dysfunction with a decrease in coronary flow reserve can occur before overt epicardial coronary artery disease[4] and has been identified as an independent prognostic factor after heart transplantation.[5]

International Society for Heart and Lung Transplantation guidelines recommend screening for CAV annually after heart transplantation.[6] There are a variety of currently available diagnostic tools available to investigate macro- and microvascular dysfunction, all of which have individual advantages and limitations.[7] Coronary angiography is used for identifying coronary artery stenosis, and this technique may be supplemented by intravascular ultrasound, optical coherence tomography, and intracoronary Doppler flow measurements.[8] However, these diagnostic tools are invasive, require radiation, and the use of nephrotoxic contrast agents, and do not assess myocardial oxygenation. Endomyocardial biopsy displays dysfunction at a cellular level, but is limited by sampling error, does not allow the overall assessment of the microvascular myocardial network, and its role for surveillance of grafts older than 6-month posttransplant is limited.[9] Finally, nuclear imaging uses radioactive tracers, and the assessment of coronary flow reserve using contrast-enhanced echocardiography[10] may be limited by acoustic windows.

Oxygenation-sensitive (OS) cardiac magnetic resonance (OS-CMR) allows the assessment of changes in myocardial oxygenation. The signal intensity (SI) in OS-CMR images is modulated by the so-called T2* effects that reduce the SI in the presence of deoxygenated hemoglobin and thus act as a marker for myocardial tissue oxygenation.[10,11] These changes reflect the modification of coronary blood flow and the coronary vascular capacity to vasodilate in the case of increased oxygen demand. Thus, neither radiation nor a contrast agent is required for OS-CMR, and breathing maneuvers could replace pharmacological vasodilators. We have previously shown that hyperventilation followed by a breath-hold (apnea) induces a significant vascular response due to changes in CO_2.[12] OS-CMR can demonstrate an attenuated regional response in the presence of coronary artery stenosis but also a more diffuse abnormality that is not associated with macrovascular disease. As shown in a previous coronary artery disease cohort, OS-CMR was attenuated in myocardial territories irrigated by recently reperfused yet nonstenotic coronary arteries, suggesting this response was indicative of microvascular injury.[13] Furthermore, in addition to assessing the myocardial function, the multiparametric nature of CMR allows for a comprehensive structural evaluation of the heart. As such, the parameters of ventricular function, and tissue characteristics like edema, regional, and diffuse fibrosis may be assessed using the same examination.

The overall objective of this study was to evaluate the changes in myocardial oxygenation as a marker for microvascular function with OS-CMR using specific breathing maneuvers to trigger some vasoactive responses in a population of long-term CTx recipients, compared with healthy controls (HC).

MATERIALS AND METHODS

In this prospective, single-center, nonrandomized, mechanistic investigation, we enrolled stable, adult (age above 18) patients, 6 or more months after orthotopic CTx. Clinical stability was confirmed by 1 of the transplant cardiologists. To assess for the presence and severity of CAV, a coronary angiogram was completed before enrollment as per best clinical practice. HC were eligible if they were free of any known cardiovascular condition or cardiovascular risk factors. HCs found to have abnormal left ventricular (LV) function or volumes by CMR were excluded from the final analyses. General exclusion criteria were known contraindications to CMR (eg, metallic implants), pregnancy, a glomerular filtration rate below 45 mL/min/1.73m^2, claustrophobia, or any clinically significant respiratory disease. All participants gave informed consent. The study was approved by the Montreal Heart Institute Ethics Committee (number 13-1444).

CMR Protocol

CMR exams were performed using a clinical 3 Tesla magnetic resonance imaging system (MAGNETOM Skyra; Siemens Healthcare, Erlangen, Germany). Detailed imaging parameters are provided in the supplementary information (Text S1, SDC, http://links.lww.com/TP/B989). All participants were asked to refrain from consuming food containing caffeine for 12 hours before the exam. The participants watched a training video before the CMR examination. The breathing maneuver protocol consisted of 60 seconds of paced hyperventilation at a rate of 30 breaths/min and a subsequent long breath-hold. During hyperventilation, the patients were monitored through live video feed and were instructed to modify their breathing pattern if the rate or depth was inadequate. After 60 seconds of hyperventilation, the patients were instructed to hold their breath in an end-expiratory position until they felt the need to breathe, which they indicated by squeezing an alarm ball.[12] During the breath-hold, OS images were acquired continuously in 2 short-axis slices. The imaging protocol also included cine images in short-axis stacks and 6 long axis views for LV function. T2-weighted short-tau inversion recovery (STIR) images were acquired in 3 short-axis views for the assessment of edema. T1 maps were acquired in the same planes before and 20 minutes after a 0.1-mmol/kg intravenous bolus of gadobutrol (Gadovist, Bayer Inc., Leverkusen, Germany). These T1 maps were subsequently used for the calculation of extracellular volume (ECV) as a marker for diffuse fibrosis. Postcontrast late gadolinium enhancement (LGE) images were also acquired for visualizing focal fibrosis/necrosis. Following the exam, all participants completed a questionnaire on their experience, including side effects.

CMR Image Analyses

CMR analyses were performed in a blinded fashion, using certified software (Circle CVI, Calgary AB, Canada). Ventricular volumes were calculated by tracing epicardial and endocardial contours at end-systole and end-diastole, including trabeculae and papillary muscles, into the myocardial mass. OS-CMR in end-systolic phases and T1 mapping images were analyzed using simplified epicardial and endocardial contours (excluding trabeculae and papillary muscles). The OS response was quantified as percent change to the beginning of the breath-hold. The time-point closest to 30 seconds into the breath-hold was used for the primary statistical analysis. The observations computed at the very end of apnea are provided in the supplementary information (SDC, http://links.lww.com/TP/B989). For

assessing interobserver variability, a second experienced reader evaluated images from 14 random CTx patients. ECV was calculated from myocardial and blood pool measurements of the native and postcontrast T1 map and hematocrit. When LGE was present, the % scar per total myocardial volume was measured using a 3 SD approach from a reference myocardial contour. From the T2 STIR images, a ratio was obtained for the myocardial signal in comparison to a reference skeletal muscle. For all myocardial sequences, measurements are reported as a single global value.

Statistical Analyses

Continuous variables are presented as mean ± SD or median (lower and upper quartile), and nominal variables are presented as counts and frequency (%). Intergroup comparisons were performed using univariate linear models for parametric variables and Kruskal-Wallis test or chi-square for nonparametric ones. Pearson's or Spearman's correlation coefficient analysis was used to determine an association between the OS response and continuous variables of interest. Interobserver reliability was determined with an intraclass correlation based on single measures using a 2-way mixed model assessing absolute agreement for global oxygenation (OS) response. Statistical analysis was performed using GraphPad Prism version 8.0 (GraphPad Software, La Jolla, CA) and the Statistical Package for Social Sciences version 25 (SPSS IBM, Chicago, IL). A *P* value of <0.05 indicated statistical significance.

RESULTS

Study Population

The study population included 46 CTx patients and 25 HC. Seven CTx patients (15%) and 2 HC (8%) were excluded from the OS-CMR analysis because of breath-hold shorter than 30 seconds or insufficient OS-CMR image quality. Clinical characteristics of the study population are presented in Tables 1 and 2. Median time since CTx was 7.6 years, with a mean recipient age of 59 ± 11 years and a graft age of 45 ± 15 years at the time of the CMR exam. Among CTx patients, 23 (50%) presented with no CAV, 17 (37%) with CAV grade 1, and 6 (13%) with CAV grade 2 and 3. A cellular rejection score was calculated according to Liang et al[14] as the sum of all rejection grades (1R = 1, 2R = 2, 3R = 3) from the biopsies divided by the total number of biopsies taken from transplant until the date of CMR. The mean rejection score for the CTx patients was <1 (0.68 ± 0.35), with only 6 (13%) patients having a mean rejection score of 1.0 or greater.

TABLE 1.

Demographic characteristics of CTx patients and healthy controls

	CTx n = 46	HC n = 25	*P*
Age (y)	59.1 ± 10.6	46.5 ± 8.1	<0.001
Gender (male)	38 (83%)	16 (64%)	0.090
Donor age (y)	35.1 ± 15.2	NA	
Donor gender (male)	30 (65%)	NA	
Time since transplant (y)	7.6 (4.3–14.5)	NA	
Graft age (y)	45.0 ± 15.3	NA	
Ischemic time (min)	122 (90–194)	NA	
Height (cm)	170 ± 10	173 ± 10	0.143
Weight (kg)	77.5 ± 16.3	78.7 ± 14.5	0.764
Body mass index (kg/m^2)	27.8 ± 8.7	26.0 ± 2.8	0.320
Systolic blood pressure (mm Hg)	115.7 ± 10.9	116.4 ± 8.2	0.784
Diastolic blood pressure (mm Hg)	74.0 ± 9.1	77.2 ± 7.5	0.164
Heart rate (bpm)	79 ± 9	68 ± 7	<0.001

Mean ± SD, median (interquartile range) or frequency, and the proportion of the group, n (%) is displayed.
CTx, cardiac transplantation; HC, healthy controls.

TABLE 2.

Clinical conditions and treatment of CTx patients

Etiology of CMP pre-CTx	
Dilated	10 (22%)
Ischemic	18 (39%)
Viral	9 (20%)
Others ¥	9 (20%)
Diabetes pre-CTx/at inclusion	8 (17%)/14 (30%)
Hypertension pre-CTx/at inclusion	10 (22%)/29 (63%)
Dyslipidemia pre-CTx/at inclusion	22 (48%)/30 (65%)
Renal Function	
Creatinine (μmol/L)	104 ± 24
Mean eGFR (mL/min/1.73m2)	63 ± 16
<30[a]	0 (0%)
30–59	15 (33%)
60–89	26 (56%)
≥90	5 (11%)
Smoking habits	3 (7%)
Mean rejection score	0.68 ± 0.35
0	1 (2%)
≤1	39 (85%)
1–≤2	6 (13%)
≥2	0 (0%)
CAV	
Grade 0 (absence)	23 (50%)
Grade 1 (mild)	17 (37%)
Grade 2 (moderate)	5 (11%)
Grade 3 (severe)	1 (2%)
Immunosuppressive regimen	
Prednisone	5 (11%)
Mycophenolic acid	39 (85%)
Cyclosporine	5 (11%)
Tacrolimus	34 (74%)
Sirolimus	12 (26%)
Cardiovascular medication	
ACE inhibitor	9 (20%)
ARB	23 (50%)
Diuretics	17 (37%)
Aldosterone antagonist	2 (4%)
Beta-blocker	12 (26%)
CCB	23 (50%)
Statins	39 (85%)

Mean ± SD or frequency and the proportion of the group, n (%) are displayed.
[a]eGFR < 45 mL/min/1.73m^2 was a predefined exclusion criterion due to the use of contrast agent.
¥ includes rhumatismal, valvular, noncompaction, hypertrophic, congenital, restrictive, and peripartum cardiomyopathies.
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CAV, cardiac allograft vasculopathy; CCB, calcium channel blocker; CMP, cardiomyopathy; CTx, cardiac transplantation; eGFR, estimated glomerular filtration rate.

TABLE 3.

CMR characteristics of CTx patients and healthy controls

	CTX n = 46	HC n = 25	P
Left ventricular function			
LV ejection fraction (%)	59 ± 7	63 ± 5	0.014
Cardiac index (L/min/m^2)	2.9 ± 0.6	3.2 ± 0.7	0.042
LV end-diastolic volume (mL)	122 ± 29	151 ± 31	<0.001
LV end-diastolic volume index (mL/m^2)	67 ± 28	78 ± 12	0.066
LV end-systolic volume (mL)	51 ± 18	57 ± 15	0.160
LV end-systolic volume index (mL/m^2)	29 ± 18	29 ± 7	1.000
LV stroke volume (mL)	71 ± 16	95 ± 19	<0.001
LV stroke volume index (mL/m^2)	39 ± 12	49 ± 7	<0.001
LV mass (g)	100 ± 28	115 ± 26	0.047
LV mass index (g/m^2)	56 ± 33	59 ± 10	0.666
Left ventricular tissue characterization			
Native T1 (ms)	1206 ± 42	1175 ± 26	0.001
Patients presenting with native T1 >1227 ms[a]	10 (21%)	–	
Extracellular volume (%)	26.7 ± 3.0	23.1 ± 2.4	<0.001
Patients presenting with ECV >27.9%[a]	13 (28%)	–	
Quantification of regional fibrosis (% of analyzed mass)	11 ± 10	–	
Patients presenting with regional fibrosis (LGE)	25 (54%)	–	
T2 STIR ratio	1.80 ± 0.31	–	
Patients presenting with T2 STIR ratio >2.0	6 (13%)	–	

Mean ± SD or frequency and the proportion of the group, n (%) are displayed for CMR measurements.
[a]The reference range for the T1 and ECV measurements were obtained from the mean ± 2SD of the presented control population.
CMR, cardiac magnetic resonance; CTX, cardiac transplantation; ECV, extracellular volume; HC, healthy controls; LGE, late gadolinium enhancement; LV, left ventricle; STIR, short-tau inversion recovery.

CMR Ventricular Function and Tissue Characterization

CMR results are presented in Table 3. Compared with HC, CTx exhibited smaller end-diastolic LV volumes, smaller ejection volumes, and lower ejection fractions. ECV as a marker for diffuse fibrosis was significantly higher in CTx patients. LGE, corresponding to areas of regional fibrosis or scar, was present in 25 (54%) of CTx patients. Areas of LGE enhancement (15, 33%) were primarily at the septal insertion points of the right ventricle, while 4 (9%) reflected subendocardial scars, 5 (11%) subepicardial scars, and 1 (2%) with a midwall pattern. LGE was present in all patients with grade 2 and 3 CAV, and additional findings in relationship with CAV status are presented in Table S1. Furthermore, 6 (13%) patients yielded a globally elevated T2 STIR ratio above 2.0.

Myocardial Oxygenation Response

The breathing maneuvers were well tolerated by all CTx patients (100% ability to perform maneuvers), while 91% were able to maintain a 30-second breath-hold. Side effects such as dizziness or chest oppression occurred in 9% of patients. Of the 3264 myocardial segments from the OS images, 3074 (94%) could be analyzed. Hyperventilation increased heart rate in both CTx (+7 ± 5 bpm; $P < 0.001$) and HC (+20 ± 11 bpm; $P < 0.001$; Figure S1, SDC, http://links.lww.com/TP/B989). There was a good interobserver agreement (intraclass correlation [ICC], 0.89; 95% CI, 0.78-0.95; $P < 0.001$). The percent change in OS signal was significantly attenuated in the CTx population compared with HC at 30 seconds into the breath-hold (2.63% ± 4.16% versus 6.40% ± 5.96%; $P = 0.010$, Figure 1). The evolution of the OS response over the breath-hold is presented in Figure 2, while some selected and representative images are illustrated in Figure 3.

The presence or absence of CAV yielded some significant effect on the OS response in CTx patients at 30 seconds ($P = 0.026$; Figures 1 and 2). Follow-up analysis for multiple comparisons demonstrated that the global OS response for patients with severe CAV (grades 2 and 3; –2.2% ± 3.6%) was significantly attenuated in comparison to CTx with minimal CAV (grade 1, 3.9% ± 2.6%;

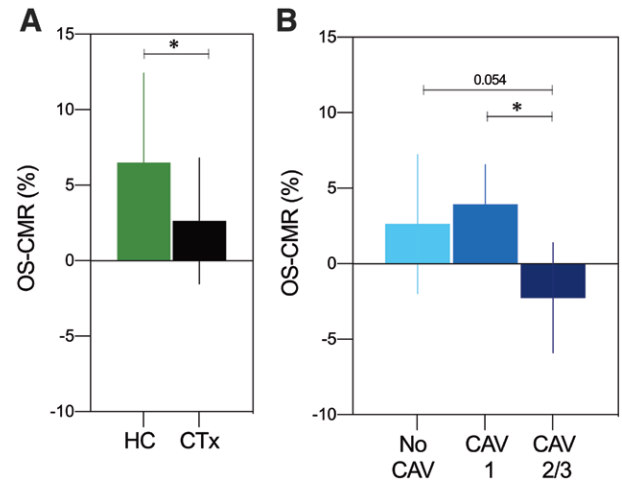

FIGURE 1. Mean ± SD are shown for the OS response to a long breath-hold. A, Healthy controls (green) had a significantly larger OS response than the CTx group (black). B, Additional analysis showed that the presence of CAV had a further attenuating impact on the OS response.*$P < 0.05$. CAV, cardiac allograft vasculopathy; CMR, cardiac magnetic resonance; CTx, cardiac transplantation; HC, healthy controls; OS, oxygenation-sensitive.

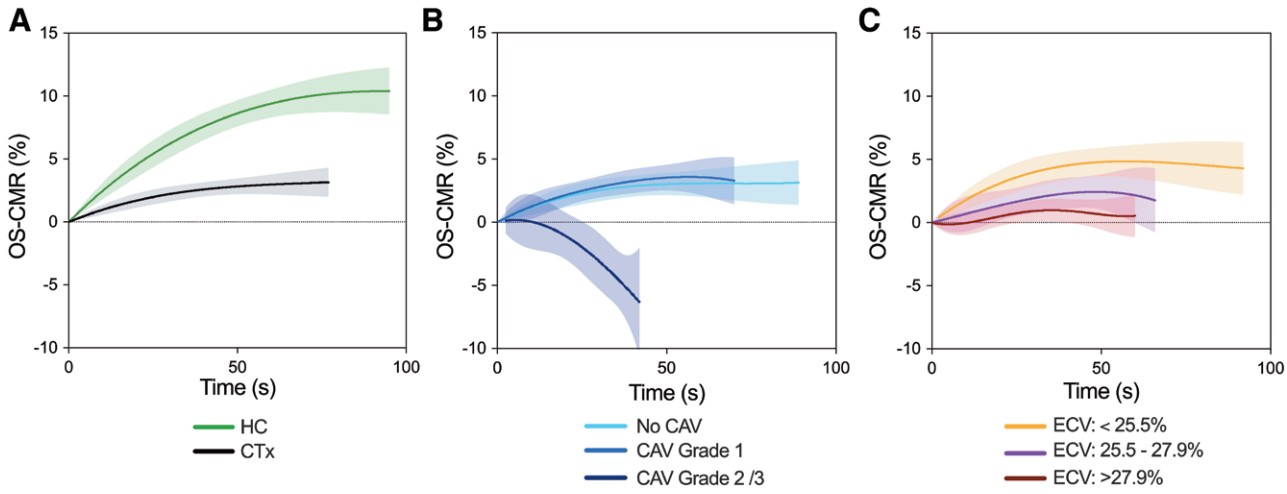

FIGURE 2. Myocardial oxygenation response to a long breath-hold. A, Global response of healthy controls (green) and all CTx patients (black). B, Patients with grade 1 CAV had a similar response as those without CAV, while those with CAV grades 2 or 3 exhibited an oxygenation deficit. Curves are truncated at the mean ± 1SD breath-hold duration of the selected patients. C, The CTx response was also categorized by extracellular volume into 3 groups, based on the range of mean ± 1SD of healthy controls ECV measurements, or between mean ± 1SD and mean ± 2SD, or greater than mean ± 2SD. The curves were cut at the mean ± 1SD end breath-hold time of the group. CAV, cardiac allograft vasculopathy; CMR, cardiac magnetic resonance; CTx, cardiac transplantation; ECV, extracellular volume; HC, healthy controls; OS, oxygenation-sensitive.

$P = 0.022$). There was a nonsignificant trend observed between the CTx recipients with severe CAV versus those without CAV (2.6% ± 4.6%; $P = 0.054$), while no difference was computed between grade 1 CAV versus no CAV ($P = 0.327$). OS responses at the very end of the breath-hold reported similar findings for the impact of CAV (Figure S2, SDC, http://links.lww.com/TP/B989). Other potential confounders, such as diabetes and dyslipidemia, did not significantly modulate the OS response (Table 4).

Association of Myocardial Oxygenation Response With Other CMR Markers

The relationships between the OS response and some structural myocardial parameters are presented in Table 5. Univariate analysis demonstrated that an attenuated OS response was associated with an increased ECV ($r = -0.352$; $P = 0.030$), a measure of diffuse myocardial fibrosis, but not with the presence of LGE. In patients without any LGE, the OS response was 3.5% ± 4.8%, 2.1% ± 3.2% in patients with insertion point enhancement, and 1.5% ± 3.7% in patients with other myocardial enhancement (midwall, subendocardial, and subepicardial; $P = 0.411$). An inverse correlation was also observed between OS-CMR and the LV end-diastolic volume index ($r = -0.445$; $P = 0.007$) and stroke volume index ($r = -0.336$; $P = 0.049$), while a nonsignificant trend was observed between the OS response and donor age ($r = -0.293$; $P = 0.070$).

DISCUSSION

In this study, we reported a markedly decreased myocardial oxygenation response in cardiac transplant patients in comparison to HC using breathing maneuvers as vasoactive stimuli. This finding was observed for both patients with and without CAV and thus was most likely due to a diminished coronary vasoreactivity. Patients with advanced CAV exhibited a more severe impairment in myocardial oxygenation response compared with patients without CAV. In addition, we observed a significant inverse relationship between the OS response and LV size as well as with the degree of diffuse myocardial fibrosis. This study showed that coronary vascular function can be safely assessed using OS-CMR in stable CTx recipients.

This investigation is clinically relevant in the context of the adverse prognosis associated with microvascular dysfunction following CTx,[5] as well as the high prevalence of asymptomatic CAV following CTx. Annual screening for myocardial ischemia is recommended, but repetitive coronary angiographies carry a risk of vascular access complications and exposure to excessive doses of radiation.[15] Several studies have investigated the changes in myocardial perfusion as a marker for coronary vascular function in relationship with the presence and the magnitude of CAV.[16,17] Noninvasive myocardial stress perfusion studies using adenosine may cause prolonged heart block in CTx recipients because cardiac denervation increases sensitivity to this drug.[18] Recently, Kazmirczak et al[19] reported the safety of stress perfusion CMR using regadenoson in CTx. They also reported that an abnormal stress perfusion was associated with a 3-year cumulative incidence of 32.1% for major adverse cardiovascular events versus 12.7% in CTx patients with a normal stress perfusion, demonstrating the benefit of testing the vascular function.[19] The high prevalence of renal insufficiency after CTx (approximately 20%) limits the use of iodinated and gadolinium-based contrast agents.[20,21] However, recent data have reported that group II gadolinium-based contrast agents are safe,[22] even in patients with stage 4 or 5 chronic kidney disease. To the best of our knowledge, our study is one of the first to investigate changes of myocardial oxygenation using a noninvasive, nonradiating, and nonpharmacological albeit developing technique.

It is well known that CO_2 affects the coronary vascular system.[23,24] More recently, it has been shown that the impact of breath-holding and hyperventilation on CO_2 allows for their use as a significant coronary vasoactive stimulus. Apnea triggers hypercapnic vasodilation, while hyperventilation induces hypocapnia and subsequent

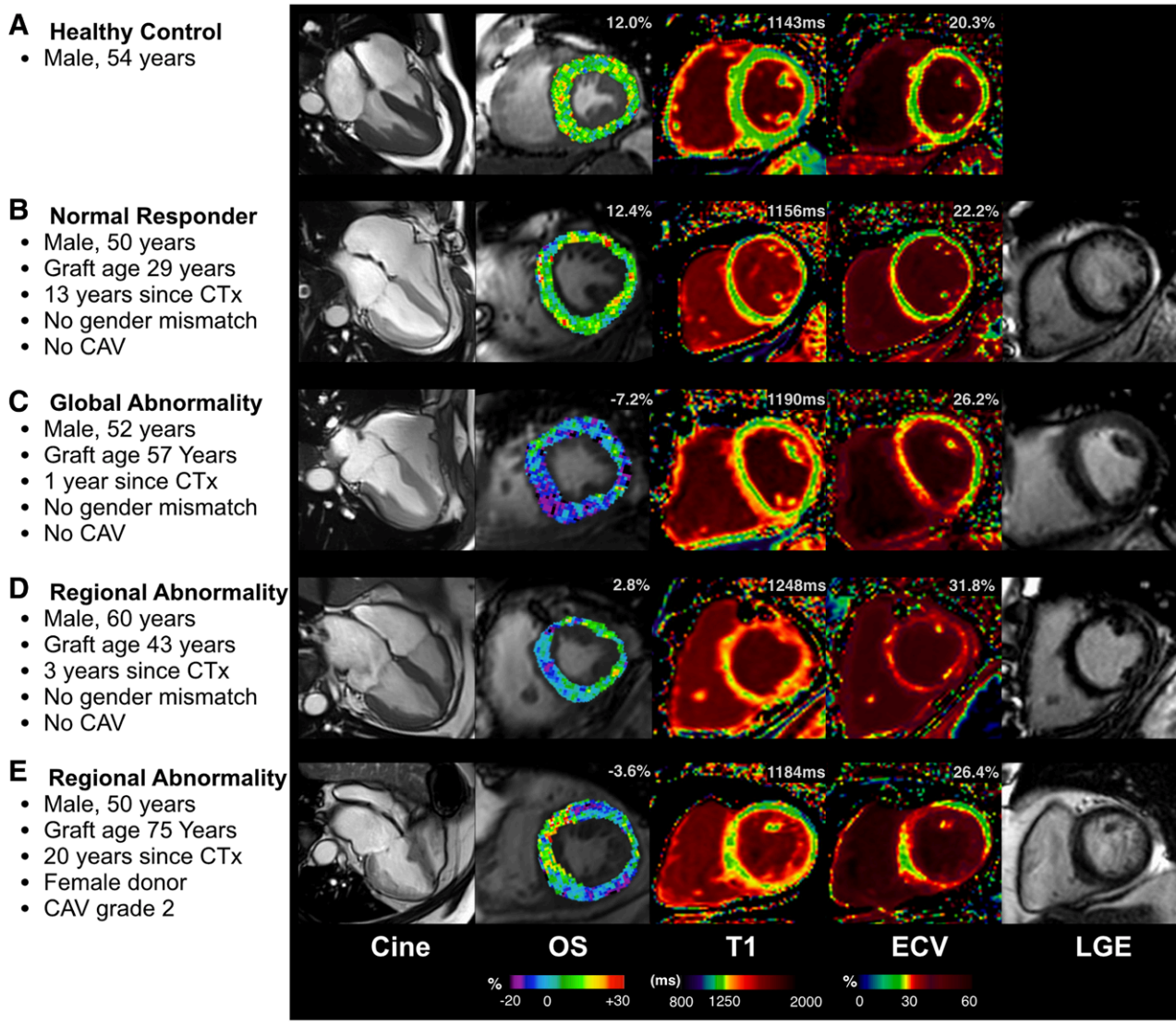

FIGURE 3. CMR results are shown of a 4-chamber cine, the myocardial oxygenation (OS) response at 30 seconds into the breath-hold in end-systole, along with a native T1 map, an ECV map, and the LGE obtained in end-diastole. A, OS, T1, and ECV from a healthy control yield values within the normal ranges (green). (B–E) demonstrate the different patterns observed in CTx patients. Patient B yielded similar results to the healthy control. While C shows a patient without CAV who had a global oxygenation abnormality, with higher ECV in the septum (red). D and E demonstrate regional OS deficits (blue) and higher native T1 and ECV (red) in the presence (E) and absence (D) of CAV. CAV, cardiac allograft vasculopathy; CMR, cardiac magnetic resonance; CTx, cardiac transplantation; ECV, extracellular volume; LGE, late gadolinium enhancement image; OS, oxygenation-sensitive.

vasoconstriction.[23,25] A previous investigation has demonstrated that an apneic stimulus could identify differences in myocardial oxygenation (T2 and T2*) between patients with hypertension and controls.[26] A combined breathing maneuvers of 60-second hyperventilation followed by spontaneous continuous breath-hold (HVBH) used here has been previously validated for this purpose in swine[27] and in healthy human volunteers,[28] obstructive sleep apnea,[29] and coronary artery disease.[13] We have focused on the 30-second time-point because the majority of patients can hold their breath for 30 seconds following proper hyperventilation.[30] This technique and the 30-second time-point have been compared with pharmacological vasodilators. In healthy volunteers, the HVBH induces greater SI changes on OS-CMR compared with intravenous adenosine.[12] The HC group in the current study yielded an increase in signal, or luxury oxygenation, while CTx recipients demonstrated an attenuation in signal, with 15% of patients having a global myocardial deoxygenation during the vasodilating HVBH stimulus indicated by a drop in signal. It is also known that hyperventilation increases heart rate[31] similar to adenosine,[12] but to a lesser extent in patients with cardiovascular disease than in age-matched controls.[32] We observed a similar trend in our CTx patients, although it is unknown how the increased heart rate modulates the OS response.

In this study, 50% of CTx recipients presented with some degree of CAV. While the attenuation of the OS response was more pronounced in advanced CAV, it was also present in patients without CAV when compared with HC. This finding is likely related to an impairment in coronary microcirculation that may occur before the development of epicardial coronary disease diagnosed on angiography.[4] Similarly, myocardial OS responses to adenosine are known to have a prognostic value in patients with chronic kidney disease,[33] and are impaired in renal transplant recipients in the absence of myocardial scarring

TABLE 4.
OS-CMR and potential confounders

	OS-CMR (Factor absent)	OS-CMR (Factor present)	*P*
Sex (female)	2.6 ± 4.5	2.9 ± 1.8	0.838
Gender mismatch	2.9 ± 4.2	1.6 ± 4.5	0.452
Hypertension	2.4 ± 5.1	2.7 ± 3.6	0.815
Diabetes	2.1 ± 4.5	4.0 ± 2.8	0.200
Dyslipidemia	2.5 ± 5.3	2.7 ± 3.5	0.914
Smoking	2.6 ± 4.3	2.9 ± 2.8	0.917

Mean ± SD OS-CMR response for when a factor is absent or present at the time of inclusion.
OS-CMR, oxygenation-sensitive cardiac magnetic resonance.

TABLE 5.
Linear association of OS-CMR in CTx

	r	*P*
Characteristics		
Recipient age	0.205	0.210
Graft age	−0.293	0.070
Time since transplant (y)	0.053	0.749
Ischemic time (min)	−0.168	0.307
eGFR	−0.057	0.730
Left ventricular function		
LV ejection fraction (%)	0.172	0.309
Cardiac index (L/min/m^2)	−0.162	0.785
LV end-diastolic volume index (mL/m^2)	−0.445	0.007
LV end-systolic volume index (mL/m^2)	−0.327	0.055
LV stroke volume index (mL/m^2)	−0.336	0.049
LV mass index (g/m^2)	0.017	0.921
Left ventricular tissue characterization		
Native T1 (ms)	−0.074	0.658
Extracellular volume (%)	−0.352	0.030
LGE extent (%)	−0.103	0.543
T2 STIR ratio	−0.137	0.419

CTx, cardiac transplantation; eGFR, estimated glomerular filtration rate; LGE, late gadolinium enhancement; LV, left ventricle; OS-CMR, oxygenation-sensitive cardiac magnetic resonance; STIR, short-tau inversion recovery.

and other potential confounders, suggesting microvascular disease in these patients as well.[34]

In this study, we reported no association between OS response and LGE, a measure of focal myocardial fibrosis. However, there was a significant relationship between OS response and ECV, a marker of diffuse myocardial fibrosis and ventricular remodeling. A previous investigation has shown that LGE was associated with an increased long-term risk for major adverse cardiac events and death in CTx recipients.[35] In the present study, LGE enhancement was not highly prevalent, with only 10 patients having myocardial enhancement beyond the ventricular insertion points. As such, the data heterogeneity and the small sample size likely explain the discrepancy in LGE findings in previous studies. On the other hand, some reports have shown that extracellular volume (ECV) is a parameter significantly associated with rejection and future cardiac events following transplantation.[36,37] Here, we reported that myocardial OS response was associated with ECV findings. These observations, and more specifically the fact that OS abnormalities were observed in patients without CAV, support the concept that OS responses most likely reflect some significant dysfunction of the microcirculation in the human cardiac allograft. Indeed, the presence of periarteriolar fibrosis on histology is a characteristic of microvasculopathy after CTx.[38] The magnitude of fibrosis and vascular endothelial growth factor increase in parallel up to 5 years after CTx followed by an isolated continuous rise in fibrosis, suggesting a relative deficit in vascular endothelial growth factor, and thus a deficient angiogenesis process in older grafts.[39,40] In addition, long-standing systemic hypertension, abnormal coronary vasomotion related to cardiac denervation, and the presence of other proatherosclerotic conditions such as the metabolic syndrome and diabetes may contribute to these findings and the progression of microvascular disease. The advantage of CMR is that the data provided by OS-CMR may be analyzed in the context of other parameters for tissue characterization and the assessment of cardiac function, thus providing a comprehensive assessment of the allograft. Nevertheless, as OS-CMR is an emerging technique, more studies are needed to confirm the association between OS imaging in response to breathing maneuvers with other techniques for the evaluation of microvascular disease.

Limitations

This investigation is the first study reporting on breathing maneuvers in combination with OS-CMR in this specific population. As such, the observations presented here could not be compared with our previous results using pharmacological-mediated vasodilation such as adenosine. Similarly, we could not validate these observations with other well-validated invasive measures of microvascular function, quantitative coronary angiography, and histology. Furthermore, the sample size was small, specifically for patients presenting with grade 2 and 3 CAV. Further studies investigating patients with more severe CAV are needed to conclude about the role of OS-CMR for the diagnosis and prognosis of CAV following CTx. Similarly, low-rejection scores were observed in this cohort and the association between rejection and OS-CMR data could not be assessed. Finally, other relevant parameters, such as the evaluation of cardiac tissue for the presence of antibody-mediated rejection and the measure of donor specific antibodies were not performed in these stable long-term patients post CTx.

The data presented here are based on the 30-second time-point assessment, which is a breath-hold duration achievable by 91% of patients. Our previous studies show that earlier time-points may still provide useful information for these patients who do not have the ability to hold their breath. T2 STIR sequences were used for the assessment of edema. This sequence has a lower reader reproducibility and is only semiquantitative relying on comparisons to reference muscle, which may have its own signal elevation. Advancements in quantitative T2 mapping may overcome the limitations posed by the T2 inversion recovery sequences applied in this study, thus allowing for a better assessment of diffuse myocardial edema. The various immunosuppressive regimens and antihypertensive drugs were not standardized, and only CTx recipients were treated with immunosuppressive medications. Some of these drugs, including rapamycin modulate

coronary vascular function. In addition, HC were significantly younger, and thus, age may have confounded our observations.

CONCLUSION

Long-term stable CTx recipients exhibit a significant attenuation in cardiac vascular reactivity in response to pCO_2 modulation as reflected by the myocardial oxygenation response. OS-CMR in combination with tissue characterization and a comprehensive cardiac imaging examination may be useful to monitor these high-risk patients and could assist clinicians in deciding the optimal timing for invasive testing posttransplantation. Additional investigations recruiting a larger number of patients with CMR performed at different time-point following CTx, and longer follow-up is needed to better understand the mechanisms involved and the long-term clinical impacts of these findings.

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REFERENCES

1. Lund LH, Edwards LB, Kucheryavaya AY, et al; International Society of Heart and Lung Transplantation. The registry of the International Society for Heart and Lung Transplantation: thirty-first official adult heart transplant report–2014; focus theme: retransplantation. *J Heart Lung Transplant.* 2014;33:996–1008.
2. Schmauss D, Weis M. Cardiac allograft vasculopathy: recent developments. *Circulation.* 2008;117:2131–2141.
3. Fearon WF, Hirohata A, Nakamura M, et al. Discordant changes in epicardial and microvascular coronary physiology after cardiac transplantation: Physiologic Investigation for Transplant Arteriopathy II (PITA II) study. *J Heart Lung Transplant.* 2006;25:765–771.
4. Haddad F, Khazanie P, Deuse T, et al. Clinical and functional correlates of early microvascular dysfunction after heart transplantation. *Circ Heart Fail.* 2012;5:759–768.
5. Hiemann NE, Wellnhofer E, Knosalla C, et al. Prognostic impact of microvasculopathy on survival after heart transplantation: evidence from 9713 endomyocardial biopsies. *Circulation.* 2007;116:1274–1282.
6. Costanzo MR, Dipchand A, Starling R, et al; International Society of Heart and Lung Transplantation Guidelines. The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant.* 2010;29:914–956.
7. Badano LP, Miglioranza MH, Edvardsen T, et al; Document Reviewers. European Association of Cardiovascular Imaging/Cardiovascular Imaging Department of the Brazilian Society of Cardiology recommendations for the use of cardiac imaging to assess and follow patients after heart transplantation. *Eur Heart J Cardiovasc Imaging.* 2015;16:919–948.
8. Nikolova AP, Kobashigawa JA. Cardiac allograft vasculopathy: the enduring enemy of cardiac transplantation. *Transplantation.* 2019;103:1338–1348.
9. Shah KB, Flattery MP, Smallfield MC, et al. Surveillance endomyocardial biopsy in the modern era produces low diagnostic yield for cardiac allograft rejection. *Transplantation.* 2015;99:e75–e80.
10. Pauling L, Coryell CD. The magnetic properties and structure of hemoglobin, oxyhemoglobin and carbonmonoxyhemoglobin. *Proc Natl Acad Sci U S A.* 1936;22:210–216.
11. Atalay MK, Reeder SB, Zerhouni EA, et al. Blood oxygenation dependence of T1 and T2 in the isolated, perfused rabbit heart at 4.7T. *Magn Reson Med.* 1995;34:623–627.
12. Fischer K, Guensch DP, Friedrich MG. Response of myocardial oxygenation to breathing manoeuvres and adenosine infusion. *Eur Heart J Cardiovasc Imaging.* 2015;16:395–401.
13. Fischer K, Yamaji K, Luescher S, et al. Feasibility of cardiovascular magnetic resonance to detect oxygenation deficits in patients with multi-vessel coronary artery disease triggered by breathing maneuvers. *J Cardiovasc Magn Reson.* 2018;20:31.
14. Liang JJ, Geske JR, Boilson BA, et al. TPMT genetic variants are associated with increased rejection with azathioprine use in heart transplantation. *Pharmacogenet Genomics.* 2013;23:658–665.
15. Lin EC. Radiation risk from medical imaging. *Mayo Clin Proc.* 2010;85:1142–1146; quiz 1146.
16. Puskás C, Kosch M, Kerber S, et al. Progressive heterogeneity of myocardial perfusion in heart transplant recipients detected by thallium-201 myocardial SPECT. *J Nucl Med.* 1997;38:760–765.
17. Hacker M, Hoyer HX, Uebleis C, et al. Quantitative assessment of cardiac allograft vasculopathy by real-time myocardial contrast echocardiography: a comparison with conventional echocardiographic analyses and [Tc99m]-sestamibi SPECT. *Eur J Echocardiogr.* 2008;9:494–500.
18. Ellenbogen KA, Thames MD, DiMarco JP, et al. Electrophysiological effects of adenosine in the transplanted human heart. Evidence of supersensitivity. *Circulation.* 1990;81:821–828.
19. Kazmirczak F, Nijjar PS, Zhang L, et al. Safety and prognostic value of regadenoson stress cardiovascular magnetic resonance imaging in heart transplant recipients. *J Cardiovasc Magn Reson.* 2019;21:9.
20. Janus N, Launay-Vacher V, Sebbag L, et al. Renal insufficiency, mortality, and drug management in heart transplant. Results of the CARIN study. *Transpl Int.* 2014;27:931–938.
21. Lachance K, White M, Carrier M, et al. Long-term evolution, secular trends, and risk factors of renal dysfunction following cardiac transplantation. *Transpl Int.* 2014;27:824–837.
22. Woolen SA, Shankar PR, Gagnier JJ, et al. Risk of nephrogenic systemic fibrosis in patients with stage 4 or 5 chronic kidney disease receiving a group II gadolinium-based contrast agent: a systematic review and meta-analysis. *JAMA Int Med*. 2019;180:223–230.
23. Neill WA, Hattenhauer M. Impairment of myocardial O2 supply due to hyperventilation. *Circulation.* 1975;52:854–858.
24. Case RB, Greenberg H. The response of canine coronary vascular resistance to local alterations in coronary arterial P CO2. *Circ Res.* 1976;39:558–566.
25. Beaudin AE, Brugniaux JV, Vöhringer M, et al. Cerebral and myocardial blood flow responses to hypercapnia and hypoxia in humans. *Am J Physiol Heart Circ Physiol.* 2011;301:H1678–H1686.
26. van den Boomen M, Manhard MK, Snel GJH, et al. Blood oxygen level-dependent MRI of the myocardium with multiecho gradient-echo spin-echo imaging. *Radiology.* 2020;294:538–545.
27. Fischer K, Guensch DP, Shie N, et al. Breathing maneuvers as a vasoactive stimulus for detecting inducible myocardial ischemia—an experimental cardiovascular magnetic resonance study. *PLoS One.* 2016;11:e0164524.
28. Guensch DP, Fischer K, Flewitt JA, et al. Breathing manoeuvre-dependent changes in myocardial oxygenation in healthy humans. *Eur Heart J Cardiovasc Imaging.* 2014;15:409–414.
29. Roubille F, Fischer K, Guensch DP, et al. Impact of hyperventilation and apnea on myocardial oxygenation in patients with obstructive sleep apnea—an oxygenation-sensitive CMR study. *J Cardiol.* 2017;69:489–494.
30. Parkes MJ. Breath-holding and its breakpoint. *Exp Physiol.* 2006;91:1–15.
31. Alexopoulos D, Christodoulou J, Toulgaridis T, et al. Hemodynamic response to hyperventilation test in healthy volunteers. *Clin Cardiol.* 1995;18:636–641.
32. Hawkins SM, Guensch DP, Friedrich MG, et al. Hyperventilation-induced heart rate response as a potential marker for cardiovascular disease. *Sci Rep.* 2019;9:17887.
33. Shah R, Parnham S, Liang Z, et al. Prognostic utility of oxygen-sensitive cardiac magnetic resonance imaging in diabetic and nondiabetic chronic kidney disease patients with no known coronary artery disease. *JACC Cardiovasc Imaging.* 2019;12:1107–1109.
34. Parnham S, Gleadle JM, Bangalore S, et al. Impaired myocardial oxygenation response to stress in patients with chronic kidney disease. *J Am Heart Assoc.* 2015;4:e002249.

35. Hughes A, Okasha O, Farzaneh-Far A, et al. Myocardial fibrosis and prognosis in heart transplant recipients. *Circ Cardiovasc Imaging*. 2019;12:e009060.
36. Patrizia P, Rimoldi O, Masciocco G, et al. Prognostic value of cardiac magnetic resonance acquired at one year after heart transplantation. *J Heart Lung Transplant*. 2019;38:S209–S210.
37. Dolan RS, Rahsepar AA, Blaisdell J, et al. Multiparametric cardiac magnetic resonance imaging can detect acute cardiac allograft rejection after heart transplantation. *JACC Cardiovasc Imaging*. 2019; 12(8 Pt 2):1632–1641.
38. Zakliczyński M, Konecka-Mrówka D, Lekston A, et al. Microvasculopathy observed in early or late endomyocardial biopsies is not related to angiographically confirmed transplanted heart coronary vasculopathy. *Transplant Proc.* 2009;41:3209–3213.
39. Gramley F, Lorenzen J, Pezzella F, et al. Hypoxia and myocardial remodeling in human cardiac allografts: a time-course study. *J Heart Lung Transplant.* 2009;28:1119–1126.
40. Vecchiati A, Tellatin S, Angelini A, et al. Coronary microvasculopathy in heart transplantation: consequences and therapeutic implications. *World J Transplant.* 2014;4:93–101.