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ORIGINAL ARTICLE

Peripheral microvascular dysfunction is associated with plaque progression and adverse long-term outcomes in heart transplant patients

Ilke Ozcan[1], Takumi Toya[1,2], Michel T. Corban[1], Ali Ahmad[1], Lilach O. Lerman[3], Sudhir S. Kushwaha[1] and Amir Lerman[1]*

[1]*Department of Cardiovascular Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55902, USA; and* [2]*Division of Cardiology, National Defense Medical College, Tokorozawa, Japan and* [3]*Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN, USA*

Abstract

Aims Cardiac allograft vasculopathy (CAV) is the major cause of increased morbidity and mortality after heart transplantation. Peripheral endothelial dysfunction (PED) is associated with early atherosclerosis and future risk of major adverse cardiovascular events (MACE) in non-heart transplant population. We aimed to investigate the association of PED with future MACE, and plaque progression assessed by intravascular ultrasound (IVUS) after heart transplantation.

Methods and results We included 66 transplant patients who underwent serial IVUS surveillance for CAV and baseline assessment of peripheral endothelial function using reactive hyperaemia peripheral arterial tonometry. PED was defined as reactive hyperaemia index < 2. The primary endpoint of the study was to investigate the association of PED with CAV progression assessed by intravascular ultrasound (IVUS). CAV progression was assessed as the change (Δ) in plaque volume divided by segment length, and Δ plaque index (plaque volume/vessel volume), adjusted for the time between IVUS measurements (median 3.0 [2.2, 3.1] years). The secondary endpoint was to investigate the association between PED and future MACE, which was defined as any incident of revascularization, heart failure hospitalization, stroke, myocardial infarction, re-transplantation, and death. Patients with PED (n = 27) had more yearly plaque progression (0.50 ± 0.66 vs. 0.15 ± 0.50 mm^3/mm/year, P = 0.02) and a higher Δ plaque index (2.41 ± 2.53% vs. 0.69 ± 2.22%, P = 0.01). Patients with PED were more likely to experience MACE during a median follow-up of 8.2 years (interquartile range [7.6, 8.4]), after adjustment for potential cofounders such as age, high-density lipoprotein cholesterol levels, total rejection score, baseline International Society for Heart & Lung Transplantation CAV grade, and indication of transplantation. (hazard ratio 2.15, 95% confidence interval [1.09, 4.23], P = 0.03).

Conclusions Peripheral endothelial dysfunction is associated with increased plaque progression and adverse long-term cardiovascular outcomes in transplant patients. PED assessment might be a useful clinical tool for risk stratification after heart transplantation.

Keywords Heart transplantation; Peripheral endothelial function; Cardiac allograft vasculopathy; Reactive hyperaemia peripheral arterial tonometry; Intravascular ultrasound

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**Correspondence to: Amir Lerman, Department of Cardiovascular Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55902, USA. Tel: +1-507-255-4152; Fax: +1-507-255-7798. Email: lerman.amir@mayo.edu*

Introduction

Advances in post-transplant treatment and immunosuppression strategies have improved long-term survival after heart transplantation; however, cardiac allograft vasculopathy (CAV) remains the leading cause of mortality following heart transplant.[1] The concentric and longitudinal distribution pattern and diffuse nature makes it challenging to diagnose asymptomatic CAV by coronary angiography; therefore, intracoronary imaging with intravascular ultrasound (IVUS)

or optical coherence tomography is useful in serial assessment of disease progression.[2] There is therefore an unmet need for stratifying high-risk heart transplant patients for CAV development noninvasively.

The exact mechanism of CAV is thought to be multifactorial.[3] Host immune response against the graft certainly has an important role in CAV progression, although not sufficient to explain the process alone. Moreover, traditional cardiovascular risk factors have also been demonstrated to promote CAV.[4] The endothelium is the primary site of damage imposed by these traditional cardiovascular risk factors, and the resultant endothelial dysfunction leads to atherosclerosis.[5] In fact, coronary endothelial dysfunction in heart transplant patients has also been shown to precede CAV and future adverse cardiovascular events.[6,7] However, the invasive nature of coronary endothelial function assessment limits its widespread use as routine follow-up procedure in heart transplant recipients. Reactive hyperaemia peripheral arterial tonometry (RH-PAT) is an FDA approved, less operator-dependent non-invasive method to assess peripheral endothelial dysfunction (PED) by measuring changes in digital pulse waveforms during reactive hyperaemia.[8] Given the systemic nature of endothelial dysfunction, peripheral endothelial function assessment using RH-PAT index (RHI) correlates with coronary endothelial dysfunction with high accuracy and can predict future risk of atherosclerotic cardiovascular disease in non-heart transplant patients.[8–11] However, prognostic values of peripheral endothelial assessment using RH-PAT for CAV and long-term outcomes are not well characterized in heart transplant patients.

We hypothesized that PED would be associated with increased CAV progression and adverse outcomes in heart transplant patients. Specifically, we aimed to investigate the relationship between PED and plaque progression assessed by IVUS, and its association with long-term outcomes in heart transplant patients.

Methods

Study design and population

This retrospective observational study was performed at a tertiary referral centre using a prospective heart transplant database. The study complies with the Declaration of Helsinki, study protocol was approved by the Mayo Clinic Institutional Review Board, and all patients provided written informed consent. Consecutive cardiac transplant patients who underwent non-invasive peripheral endothelial function testing on the day of their routine annual coronary angiography and IVUS procedures between 2011 and 2012 were included in the study. The primary endpoint of the study was to observe the association between PED and CAV progression, whereas the secondary endpoint was the association between PED and major adverse cardiovascular events during long-term follow-up.

Coronary angiography and intravascular ultrasound analysis

Coronary angiography was performed according to the standard protocol, and CAV was angiographically categorized using the International Society for Heart & Lung Transplantation (ISHLT) guidelines.[12]

Intravascular ultrasound studies are performed on the patients on a yearly basis with coronary angiography, as part of CAV surveillance. The methods for acquisition and analysis of IVUS images have been previously described.[13,14] Briefly, after intracoronary administration of nitroglycerin, a 20 MHz, 2.9 F phased-array IVUS catheter (Eagle Eye Gold, Volcano Corporation/Philips, Rancho Cordova, CA, USA) was advanced into the middle left anterior descending artery, and automatic pull-back to the coronary ostium at a speed of 0.5 or 1.0 mm/s was performed during the electrocardiogram-gated image acquisition at the peak of the R wave. Offline volumetric analysis of IVUS data was performed using Echo Plaque (Version 4.3.12, INDEC Systems Inc, Santa Clara, CA) by investigators who were blinded to RHI results and clinical data. Measurements were performed starting with the first complete vascular ring distal to the bifurcation of the left circumflex artery lumen, and plaque volume (PV) and vessel volume (VV) were analysed in the proximal part of middle left anterior descending artery. The semi-automated contour detection feature of the software was used after manual detection of frames in intervals of two to four frames, depending on image heterogeneity. Border detection was corrected manually in all frames afterwards. The Simpson rule for volumetric measurement was used. Each measurement was normalized to the examined segment length (mm^3/mm). Plaque index was calculated as: (plaque volume/vessel volume) × 100%. Changes in plaque, lumen, and vessel volume or plaque index were defined as absolute change in mm^3 for volumes, and percentage for indices, calculated as follow-up minus baseline values (Δ) and adjusted for the time between baseline and follow-up IVUS measurements.

Assessment of peripheral endothelial function

Peripheral endothelial function was assessed by RHI using the EndoPAT 2000 device (Itamar Medical Inc., Caesarea, Israel) at the time of baseline IVUS, as previously described.[10,15] Briefly, finger probes were placed on the middle finger on both hands of the subject. After 5 minutes of baseline measurement, a blood pressure cuff around the test arm was inflated with a pressure of 60 mmHg above baseline

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systolic blood pressure, up to 200 mmHg for 5 min. This was followed by deflation of the cuff, and 6 min peripheral arterial tonometry (PAT) measurement. Control arm (contralateral arm) was free of occlusion during the procedure. RH-PAT ratio was determined in both arms, by dividing the average pulse wave amplitude after pressure cuff deflation, by the average during the baseline period. RHI was then calculated automatically with an algorithm that normalizes the baseline signal and indexes the RH-PAT ratios of the test and the control arms. All vasoactive medications, including calcium channel blockers, β-blockers, and long-acting nitrates, were discontinued for at least 24 h before endothelial function testing. PED was defined as RHI < 2.0, in line with previous studies.[10,15,16]

Endomyocardial biopsies

Endomyocardial biopsies are routinely performed every month during the first year according to our institutional protocols, as previously described.[17] All biopsies were graded according to the ISHLT 2005 guidelines, for cellular and antibody-mediated rejection.[18] A 1 year total rejection score was calculated.[17] Biopsy grades were considered 0R as 0, 1R as 1, 2R as 2, 3R as 3 and the cumulative scores divided by the total number of biopsies taken in the first-year post heart transplantation.

Assessment of outcome events

All clinical data were collected by undertaking a detailed review of medical records to determine the adverse events during follow-up. Information was collected to detect the following events: myocardial infarction, coronary revascularization, hospitalization for heart failure, cerebrovascular event (haemorrhagic or ischaemic stroke, and transient ischaemic attack), re-transplantation, and death. Cause of death was determined by reviewing death certificates, autopsy reports, and phone interviews, when available. Composite major adverse cardiovascular events (MACE) were defined as any incident of myocardial infarction, coronary revascularization, heart failure hospitalization, cerebrovascular event, re-transplantation, and death. Individual events were adjudicated and confirmed by two independent investigators.

Statistical analysis

Normally distributed continuous parameters were presented as mean ± standard deviation and compared by Student's *t*-test. Non-normally distributed continuous parameters were presented by median (interquartile range) and compared by nonparametric Wilcoxon rank-sum test. Shapiro–Wilk test was used to assess normal distribution. Categorical data were presented as number (%) and compared between groups with the χ^2 test (or Fisher's exact test). Linear regression analyses were performed to identify correlations between two parameters. Skewed data were log-transformed (Ln) to follow normal distribution. Then, the associations between parameters were assessed using Pearson's correlation test. Multiple regression analyses were performed to estimate the effects of covariates on plaque progression. Univariable and multivariable Cox proportional hazard models were used to estimate the effect of PED on adverse outcomes. Multivariable analyses were conducted with a step-wise addition strategy, by incorporating factors that showed a trend of difference ($P < 0.10$) in the univariable models.[19] Kaplan–Meier methods were used to assess MACE-free survival rates. Only the first event was used for the MACE analyses in patients with multiple events. The difference between groups was analysed using the log-rank test. A two-tailed P value < 0.05 was considered statistically significant. All statistical analyses were conducted using the JMP Pro software version 14.1.0 (SAS Institute, Inc, Cary, NC).

Results

Baseline characteristics

A total of 67 patients who underwent non-invasive peripheral endothelial function testing were included. One patient who did not have an available baseline IVUS was excluded from the study, leaving a total of 66 patients in the analyses. Baseline characteristics of the whole group and classified by RHI at the time of PAT/baseline IVUS study are presented in *Table 1*. The mean age of the overall group was 56 ± 14 years, and 76% were male patients. The mean RHI was 2.2 ± 0.59. Of 66 patients, 27 patients (41%) had PED defined as RHI < 2 at baseline. The median duration between transplantation and baseline IVUS/PAT study was 5.5 (2.0, 8.0) years and did not differ between groups (4.9 [2.0, 8.0] vs. 5.9 [2.1, 9.9] years, $P = 0.47$).

Age (55 ± 15 vs. 56 ± 14 years, $P = 0.81$) and sex (male, 21 [78%] vs. 29 [74%], $P = 0.75$) of the patients were similar between groups. Heart failure aetiology was not significantly different ($P = 0.70$). Patients with ischaemic compared with non-ischaemic cardiomyopathy had similar RHI (2.16 ± 0.6 vs. 2.20 ± 0.59, $P = 0.80$). Donor age was younger in patients with RHI < 2 than those with RHI ≥ 2 (29 ± 11 vs. 36 ± 14 years, $P = 0.04$). Patients with RHI < 2 were more likely to have diabetes mellitus (13 [48%] vs. 9 [23%], $P = 0.03$), and tended to have lower levels of high-density lipoprotein cholesterol (50 ± 13 vs. 57 ± 18 mg/dL, $P = 0.06$). There were no other significant differences between groups (*Table 1*).

Table 1 Baseline characteristics

		RHI		
	Total (n = 66)	<2 (n = 27)	≥2 (n = 39)	P value
RHI	2.2 ± 0.59	1.6 ± 0.28	2.6 ± 0.41	<0.0001
Recipient age (years)	56 ± 14	55 ± 15	56 ± 14	0.81
Male, n (%)	50 (76%)	21 (78%)	29 (74%)	0.75
Time from transplant to RH-PAT (years)	5.5 (2.1, 8.3)	4.9 (2.0, 8.0)	5.9 (2.1, 9.9)	0.47
Time between IVUS studies (years)	3.0 (2.2, 3.1)	2.9 (2.2, 3.1)	3.0 (2.8, 3.1)	0.42
Body mass index (kg/m^2)	28.3 ± 4.5	28.1 ± 4.6	28.3 ± 4.6	0.84
Hypertension, n (%)	37 (56%)	17 (63%)	20 (51%)	0.35
Systolic blood pressure (mmHg)	122 ± 15	121 ± 13	123 ± 16	0.56
Diastolic blood pressure (mmHg)	79 ± 10	79 ± 9	79 ± 11	0.89
ACE-I or ARB, n (%)	24 (36%)	12 (44%)	12 (31%)	0.26
Calcium channel blockers, n (%)	16 (25%)	8 (31%)	8 (21%)	0.38
Diabetes mellitus, n (%)	22 (33%)	13 (48%)	9 (23%)	0.03
Indication for heart transplant, (ICM/DCM/Other), n	(20/20/26)	(9/9/9)	(11/11/17)	0.70
Donor age, years (n = 64)	33 ± 13	29 ± 11	36 ± 14	0.04
Ischaemic time, minutes (n = 55)	185 ± 40	188 ± 35	182 ± 44	0.57
CMV viremia, n (%)	14 (21%)	4 (15%)	10 (26%)	0.29
Primary immunosuppression, n (%)				0.82
Calcineurin inhibitor	28 (42%)	11 (39%)	17 (44%)	
Sirolimus	38 (58%)	16 (60%)	22 (56%)	
Secondary immunosuppression, n (%)				
Azathioprine	16 (24%)	7 (26%)	9 (23%)	0.79
Mycophenolate mofetil	47 (71%)	20 (74%)	27 (70%)	0.67
Prednisone, n (%)	35 (53%)	13 (48%)	22 (56%)	0.51
Statins, n (%)	60 (91%)	24 (89%)	36 (92%)	0.64
Anti-platelet agents, n (%)	19 (29%)	6 (22%)	13 (33%)	0.33
Glucose (mg/dL)	104 (93, 127)	108 (93, 138)	102 (92, 116)	0.24
Total cholesterol (mg/dL)	206 ± 61	209 ± 77	203 ± 48	0.71
HDL-C (mg/dL)	54 ± 17	50 ± 13	57 ± 18	0.06
LDL-C (mg/dL)	108 ± 46	110 ± 59	106 ± 35	0.78
Triglycerides (mg/dL)	184 (111, 261)	186 (135, 301)	163 (107, 251)	0.41
Creatinine (mg/dL)	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	0.45
eGFR (mL/min/1.73 m^2)	57 (47–76)	52 (47, 76)	61 (50, 76)	0.55
LVEF (%)	63 ± 5.8	63.2 ± 4.9	62.3 ± 6.3	0.53
Rejection score at baseline, n (%) (n = 65)				0.50
0R	51 (78%)	21 (80%)	30 (77%)	
1R	12 (18%)	5 (19%)	7 (18%)	
2R	2 (3%)	0 (0%)	2 (5%)	
Total rejection score (n = 64)	0.34 (0.09, 0.55)	0.38 (0.17, 0.63)	0.28 (0.07, 0.54)	0.19
ISHLT CAV grade at baseline, n (%)				0.79
Grade 0	26 (40%)	10 (37%)	16 (41%)	
Grade 1	36 (55%)	16 (59%)	20 (54%)	
Grades 2–3	4 (6%)	1 (4%)	3 (7%)	

ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CMV, cytomegalovirus; DCM, dilated cardiomyopathy; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; ICM, ischaemic cardiomyopathy; ISHLT, international society for heart & lung transplantation; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; RHI, reactive hyperaemia peripheral arterial tonometry index.

RH-PAT index, rejection, and immunosuppression

There were no significant associations between PED and rejection. Baseline cellular rejection grades (P = 0.50) as well as the first-year total rejection scores were similar between patients with RHI < 2 vs. RHI ≥ 2. (0.38 [0.17, 0.63] vs. 0.28 [0.07, 0.54]; P = 0.19). Incidence of antibody-mediated rejection episodes was also not significantly different (2 [8%] vs. 4 [10%], P = 0.73).

Type of primary immunosuppression did not differ significantly between groups (P = 0.82). Twelve patients (18%) had converted to sirolimus from calcineurin inhibitor within 1 year prior to baseline studies. Of these, eight patients had RHI < 2.

RH-PAT index on plaque progression

Baseline IVUS characteristics are summarized in *Table 2*. Baseline plaque volume and plaque index were similar between groups (4.6 [2.4, 8.5] vs. 5.2 [2.9, 7.9] mm^3/mm, P = 0.54 for plaque volume; 30.4 [16.4, 40.9] vs. 34.5 [21.3, 44.8] %, P = 0.22 for plaque index). The median time between baseline and follow-up IVUS analyses was 3.0 [interquartile range 2.2, 3.1] years and did not differ between patients with and without PED (2.9 [2.2, 3.1] vs. 3.0 [2.8, 3.1], P = 0.42). RHI was inversely correlated with Δ plaque volume (r = −0.27, P = 0.03). This correlation remained significant after adjustment for recipient age, sex, low-density lipoprotein cholesterol levels, and diabetes mellitus (standardized β = −0.27, t

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Table 2 Baseline intravascular ultrasound characteristics

	Total (n = 66)	RHI		P value
		<2 (n = 27)	≥2 (n = 39)	
PV/SL (mm^3/mm)	4.9 (2.7, 8.0)	4.6 (2.4, 8.3)	5.2 (2.9, 7.9)	0.54
VV/SL (mm^3/mm)	15.4 (13.1, 19.4)	15.2 (13.8, 20.3)	15.6 (12.6, 19.0)	0.58
LV/SL (mm^3/mm)	10.9 (7.7, 13.1)	12.5 (8.6, 13.5)	9.6 (7.4, 12.7)	0.14
Plaque index (%)	33.4 (20.6, 44.7)	30.4 (16.4, 40.9)	34.5 (21.3, 44.8)	0.22

LV, lumen volume; PV, plaque volume; RHI, reactive hyperaemia peripheral arterial tonometry index; SL, segment length; VV, vessel volume

ratio = −2.2, P = 0.03) (*Table 3*) Patients with RHI < 2 had significantly more plaque progression (0.50 ± 0.66 vs. 0.15 ± 0.50 mm^3/mm/year, P = 0.02) (*Figure 1A*) and Δ plaque index (2.41 ± 2.53 vs. 0.69 ± 2.22%, P = 0.01) (*Figure 1B*).

RH-PAT index on cardiovascular outcomes

The median follow-up duration after baseline studies was 8.2 (interquartile range [7.6, 8.4]) years and did not differ between groups (8.2 [7.3, 8.3] vs. 8.2 [7.8, 8.6], P = 0.40). In total, 38 patients had composite MACE (myocardial infarction/stroke/heart failure hospitalization/revascularization/re-transplantation/all-cause death). In the whole group, 15 patients underwent revascularization for CAV, 13 were admitted due to heart failure, 2 had myocardial infarction, 5 had a cerebrovascular event, and 2 patients underwent re-transplantation. Eighteen patients died, with 6 determined to have a cardiovascular event as the likely cause of death. The cause of death could not be identified in 4 patients.

On survival analysis, patients with RHI < 2 tended to have lower MACE-free survival than those with RHI ≥ 2 (log-rank P = 0.05) (*Figure 2*), with a borderline increased risk of composite MACE [hazard ratio 1.86, 95% confidence interval (CI) (0.99, 3.53), P = 0.06] (*Table 4*). However, after adjustment for potential cofounders which tended to have an association with composite MACE in univariable Cox proportional hazard analyses, as shown in *Table 4* (age, high-density lipoprotein cholesterol levels, first-year total rejection score, baseline angiographic ISHLT CAV grade, and ischaemic cardiomyopathy), RHI < 2 was significantly associated with an increased risk of composite MACE (hazard ratio 2.15, 95% CI [1.09, 4.23], P = 0.03) (*Table 4*).

Table 3 Multivariable linear regression analyses for plaque progression

	Std β	t ratio	P value
RHI	−0.27	−2.2	0.03
Age	0.08	0.63	0.53
Sex, male	0.12	0.92	0.36
LDL-C	−0.08	−0.67	0.51
Diabetes mellitus	0.10	0.79	0.43

CI, confidence interval; LDL-C, low density lipoprotein cholesterol; RHI, reactive hyperaemia peripheral arterial tonometry index; Std, standardized.

Discussion

Our study demonstrates that PED is associated with progression of coronary allograft vasculopathy assessed with IVUS and an increased risk of major adverse cardiovascular events during a long-term follow-up in heart transplant patients. The study further supports the potential role of non-invasive assessment of endothelial function as a tool to stratify high-risk patients following heart transplantation.

Endothelial dysfunction plays a fundamental role in the pathogenesis of atherosclerotic disease. The endothelium has many roles in maintaining homeostasis, from regulation of blood flow and vascular tone, to anti-thrombotic and anti-inflammatory activities, with nitric oxide (NO) as the major compound responsible for these functions.[5] Any metabolic disturbance in the blood flow that might cause a subsequent alteration in NO activity therefore result in a pro-thrombotic, vasoconstrictive, and inflammatory state, in favour of atherosclerotic plaque formation. Endothelial dysfunction is not only the initiating factor but is also associated with vulnerable plaque characteristics and accelerated progression.[20,21] As endothelial cells line the vessels of the entire body and are under similar influences, endothelial dysfunction can be thought of as a systemic disease. Indeed, peripheral and intracoronary-assessed endothelial dysfunction correlate well,[9] and both are associated with cardiac, cerebrovascular, and peripheral events.[10,22,23] Although plaque progression has additional local components, such as shear stress[24] and immune damage,[17] PED could represent the culmination of risk factors and impaired vascular repair capacity in an individual. Furthermore, microvascular dysfunction caused by these factors could influence the local hemodynamics.[25]

The systemic nature of endothelial dysfunction following heart transplantation is unclear. Coronary endothelial dysfunction was shown to precede plaque progression in transplant patients.[6] However, the studies on peripheral endothelial function after transplantation are limited, and mostly focus on effect of heart transplantation on the

Figure 1 Progression of CAV in the two groups. Patients with peripheral endothelial dysfunction (RHI < 2) had significantly more plaque progression (Δ Plaque volume) (A) and higher changes in plaque index (Δ Plaque index) (B) during a follow-up of median 3.0 [interquartile range 2.2, 3.1] years, compared with patients with normal peripheral endothelial function (RHI ≥ 2). CAV, coronary allograft vasculopathy; RHI, reactive hyperaemia peripheral arterial tonometry index.

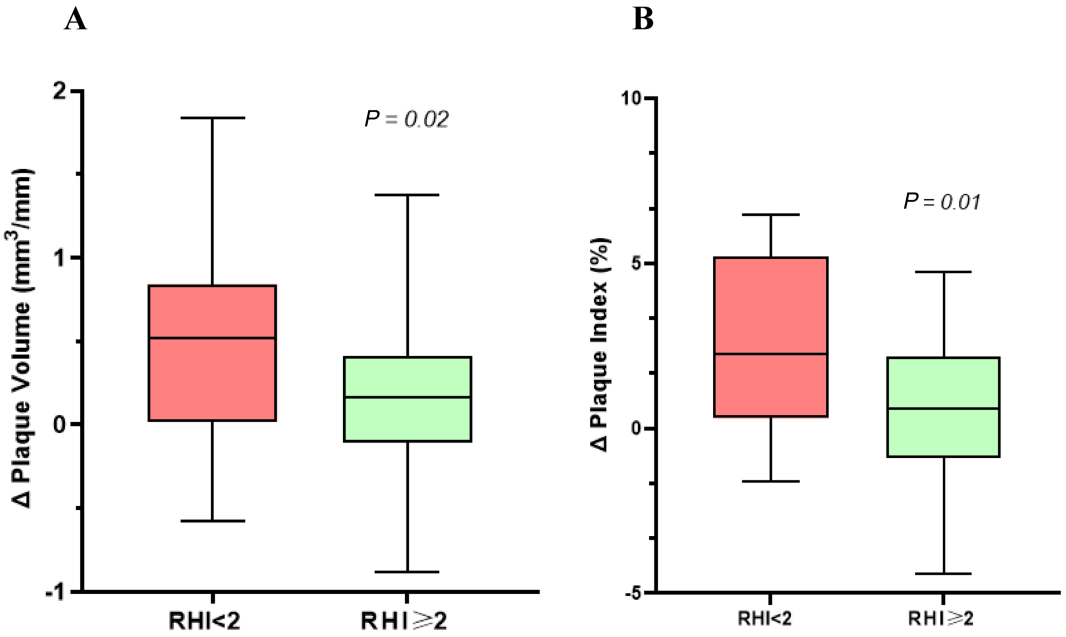

Figure 2 Comparison of MACE-free survival between patients with normal vs. abnormal RHI. Comparison of Kaplan–Meier curves for incidence of composite MACE (myocardial infarction/stroke/heart failure hospitalization/revascularization/re-transplantation/all-cause death) between patients with RHI ≥ 2 vs. <2. Patients with peripheral endothelial dysfunction (RHI < 2) tended to have lower MACE-free survival compared with those with RHI ≥ 2. (Log-rank *P* = 0.05). MACE, major adverse cardiovascular events; RHI, reactive hyperaemia peripheral arterial tonometry index.

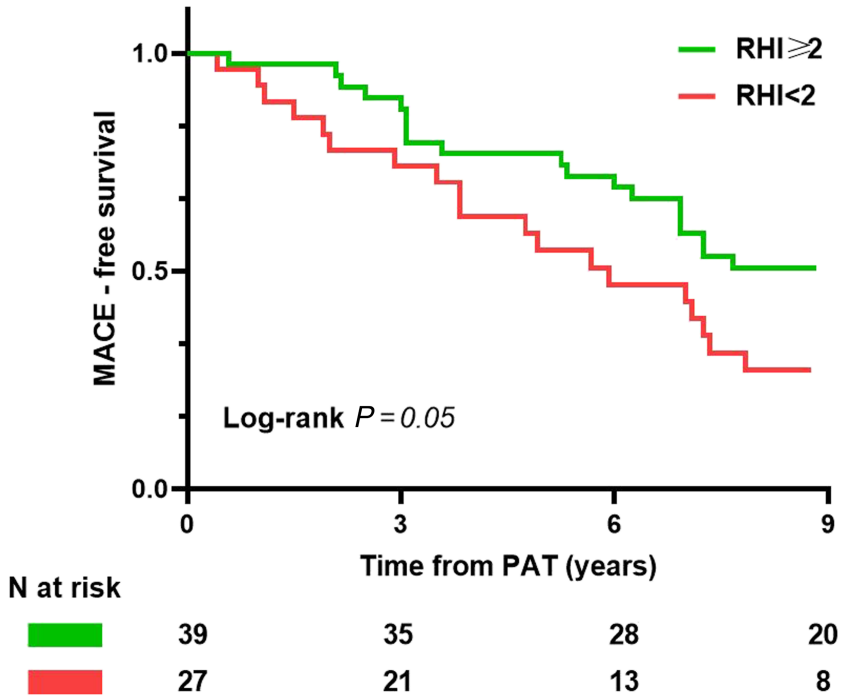

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Table 4 Univariable and multivariable Cox proportional hazard ratio analyses for composite MACE

	Univariable		Multivariable model	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
RHI < 2	1.86 (0.99, 3.53)	0.06	2.15 (1.09, 4.23)	0.03
Age	1.02 (0.99, 1.05)	0.07	1.03 (1.00, 1.06)	0.05
HDL-C	0.97 (0.95, 0.99)	0.03	0.98 (0.95, 1.01)	0.16
Total rejection score[a] (per unit)	2.79 (0.81, 9.43)	0.10	2.54 (0.60, 10.73)	0.20
Baseline ISHLT grade[b]	1.89 (0.99, 3.61)	0.05	1.13 (0.48, 2.66)	0.79
Indication for transplantation[c]	2.03 (1.06, 3.90)	0.04	1.02 (0.47, 2.24)	0.96

CI, confidence interval; HDL-C, high density lipoprotein cholesterol; HR, hazard ratio; ISHLT, international society for heart & lung transplantation; MACE, major adverse cardiovascular events; RHI, reactive hyperaemia peripheral arterial tonometry index.
[a]Calculated as sum of ISHLT grades of rejection divided by the total number of biopsies during the first year after heart transplantation
[b]Baseline angiographic ISHLT CAV grade ≥ 1.
[c]Ischaemic vs. non-ischemic

peripheral endothelium.[26,27] Roig *et al*. have previously demonstrated that patients with normal flow-mediated dilatation (FMD) of brachial artery at 1 month after transplantation had a lower probability of developing CAV in the first year compared with the patients with abnormal FMD.[28] The current study extended this previous observation and demonstrates the association between plaque progression and PED beyond the first month after transplantation. We have also assessed plaque progression quantitatively and prospectively in our study group, rather than dichotomizing our findings as presence or absence of CAV. Our methods differ in terms of PED assessment, as FMD is based on macrovascular reactivity to shear stress, while PAT reflects microvascular function through both endothelial-dependent and independent mechanisms.[9] As large vessel endothelial dysfunction presumably follows microvascular dysfunction,[25] PAT assessment might be more useful in demonstrating early dysfunction. Importantly, in the current study, we further demonstrated that those with PED had an increased risk of MACE during a long-term follow-up, independent of potential confounders. Thus, it is possible that similar to the non-transplant population, endothelial dysfunction represents the integration of multiple conventional and non-conventional risk factors that may contribute to events. This finding, which translates the previously shown association of PED and allograft vasculopathy to long-term outcomes, could further support the systemic nature of endothelial dysfunction after heart transplantation.

Pathophysiology of CAV is complex and incompletely understood. We have previously demonstrated that vulnerable plaque characteristics,[29] which are associated with endothelial dysfunction in non-transplant population,[20,21] are not uncommon findings in heart transplant patients. Another finding was layered fibrotic plaques that are suggestive of repeated thrombosis and subsequent plaque formation.[30] Considering the accelerated nature of CAV, these mechanisms could be implicated in the disease process. Repeated thrombosis and subsequent healing response is most probably caused by dysfunctional NO activity, and the resultant pro-thrombotic milieu.

Rejection is also associated with accelerated CAV progression.[17] Although PED is reportedly associated with inflammation in transplant patients as well, we did not observe a significant difference between groups in terms of rejection. However, the total rejection score was calculated within the first year, as biopsies are standardized during that period. Considering only a small number of patients were within the first 2 years after transplantation, first-year rejection score might not be reflective of the inflammatory state after a certain period. It is also not clear if low-grade (1R) rejection results in any systemic inflammatory state.

Sirolimus-based immunosuppression has been shown to be associated with preserved endothelial function compared with cyclosporine,[31] along with attenuation of CAV.[14] Although we did not see a difference in RHI and the type of immunosuppression that is used, almost 20% of the patients underwent sirolimus conversion within 1 year prior of baseline studies, and this might have affected our findings. Other medications and lifestyle interventions that can ameliorate endothelial dysfunction, such as statins, angiotensin-converting enzyme-inhibitors, glycaemic control, and exercise have also been associated with favourable outcomes in transplant patients. Thus, early initiation of these interventions might be particularly beneficial in patients with endothelial dysfunction.

Our study has some limitations worth noting. First, baseline studies were performed at different periods after transplantation depending on the patients. However, the current study demonstrated the yearly plaque progression, which is independent of the time of the initial study. Second, while the time between baseline and follow-up were not significantly different among patients, plaque progression was assessed as linear over time to compensate for the time differences between the two analyses. Third, only one vessel was imaged. However, considering the diffuse nature of CAV, it is reasonable to assume that the progression of the disease is similar in the remaining coronary vascular beds. Finally, although we have demonstrated a significant association of PED with MACE in a multivariable model with potential confounders, our study fell short of demonstrating

a direct association with MACE in a univariable model. Together with the mentioned limitations of our study, studies with larger patient populations are warranted to further confirm our observations.

In conclusion, systemic PED is associated with progression of CAV and increased risk of adverse cardiovascular events in heart transplant patients on long-term follow-up. Non-invasive assessment of peripheral endothelial function might be a useful tool for risk stratification in heart transplant patients.

Conflict of interest

Prof Amir Lerman declared consulting for Itamar Medical (Caesarea, Israel). The remaining authors have no conflicts of interest to report.

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None.

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