

Clinical and Functional Correlates of Early Microvascular Dysfunction After Heart Transplantation

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Background—Microvascular dysfunction is emerging as a strong predictor of outcome in heart transplant recipients. At this time, the determinants and consequences of early microvascular dysfunction are not well established. The objective of the study was to determine the risk factors and functional correlates associated with early microvascular dysfunction in heart transplant recipients.

Methods and Results—Sixty-three heart transplant recipients who had coronary physiology assessment, right heart catheterization, and echocardiography performed at the time of their first annual evaluation were included in the study. Microvascular dysfunction was assessed using the recently described index of microcirculatory resistance. The presence of microvascular dysfunction, predefined by an index of microcirculatory resistance >20 , was observed in 46% of patients at 1 year. A history of acute rejection and undersized donor hearts were associated with microvascular dysfunction at 1 year, with odds ratio of 4.0 (1.3–12.8) and 3.6 (1.2–11.1), respectively. Patients with microvascular dysfunction had lower cardiac index (3.1 ± 0.7 versus 3.5 ± 0.7 L/min per m^2 ; $P=0.02$) and mild graft dysfunction measured by echocardiography-derived left and right myocardial performance indices ($[0.54\pm 0.09$ versus 0.43 ± 0.09 ; $P<0.01$] and $[0.47\pm 0.14$ versus 0.32 ± 0.05 ; $P<0.01$], respectively). Microvascular dysfunction was also associated with a higher likelihood of death, graft failure, or allograft vasculopathy at 5 years after transplant (hazard ratio, 2.52 [95% CI, 1.04–5.91]).

Conclusions—A history of acute rejection during the first year and smaller donor hearts were identified as risk factors for early microvascular dysfunction. Microvascular dysfunction assessed using index of microcirculatory resistances at 1 year was also associated with worse graft function and possibly worse clinical outcomes. (*Circ Heart Fail.* 2012;5:759-768.)

Key Words: coronary physiology ■ microcirculation ■ coronary artery disease ■ heart transplantation ■ heart function

Cardiac allograft vasculopathy (CAV) is the leading cause of late graft failure and mortality in heart transplantation.^{1,2} Based on the registry of the International Society for Heart and Lung Transplantation, CAV is detected angiographically in 32% of patients within 5 years of transplantation.² CAV is a progressive disorder that may affect both the epicardial vessels and the microcirculation of the heart.¹⁻³

Although earlier studies on CAV have focused on epicardial disease, recent studies have called attention on the importance of microvascular disease.⁴⁻⁸ Functionally, microvascular disease is measured using coronary flow reserve (CFR with usual threshold <2.5) or the recently described index of microcirculatory resistance (IMR).^{3,9-14} Compared with CFR, IMR is a more specific and a more reproducible measure of microvascular function.¹⁵ We have previously shown that IMR improves significantly during the first posttransplant year, whereas fractional flow reserve (FFR), a marker of epicardial coronary physiology, worsens.³

At this time, the determinants and functional consequences of early microvascular dysfunction defined using the IMR have not been established. For this study, we sought to determine whether a history of acute rejection during the first posttransplant year would be a strong determinant of early microvascular dysfunction. Our second objective was to determine whether the presence of microvascular dysfunction was associated with worse ventricular function at 1 year. Finally, in an exploratory analysis, we sought to determine whether the presence of early microvascular dysfunction would be associated with a higher likelihood of death, retransplant, or early allograft vasculopathy.

Methods

Study Design

This study is a retrospective cohort study designed to determine the determinants and clinical correlates of microvascular dysfunction in heart transplant recipients. Patients enrolled in the study were transplanted between January 2001 and June 2008 at Stanford University Medical Center. Informed consent was obtained from all patients

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according to Stanford Medical Center's Human Investigation Review Board before coronary physiology measurements. Patients included in the analysis were enrolled as part of National Institutes of Health-funded trials (1 K23 HL072808-01A1, 1 PO1-AI50153, and 5 R01 HL093475-02). Data collection was performed by 2 research associates trained in data extraction.

Patient Sample

The study sample consisted of 63 adult heart transplant patients who underwent assessment of microvascular function and echocardiography as part of their first annual posttransplant evaluation at Stanford University Medical Center.

Patients with evidence of acute rejection, significant CAV, or graft failure at the time of their annual evaluation were excluded from the study. Acute rejection was defined as an event that led to an acute augmentation of immunosuppression.¹⁶ Both cellular (International Society for Heart and Lung Transplantation grade $\geq 2R$) and noncellular rejections with hemodynamic compromise (decrease in relative left ventricular ejection fraction [LVEF] $>25\%$) were considered significant rejections. Graft failure was diagnosed if patients met the Framingham Criteria for Congestive Heart Failure and required hospitalization for heart failure management.¹⁷ We excluded acute rejections at the time of the annual evaluation to allow analysis of the relationship between microvascular dysfunction and graft function, independently of an acute rejection episode. Significant epicardial CAV was predefined by a luminal stenosis $>50\%$ by quantitative angiography.¹⁸ Of the 67 patients considered for the study, 4 patients were excluded for the following reasons: 2 patients had evidence of acute cellular rejection, 1 patient had evidence of graft failure without evidence of rejection, and 1 patient had evidence of significant epicardial disease with 60% luminal stenosis in the left anterior descending artery.

The study sample represents 19% of adult patients transplanted during the study period ($n=337$). Compared with patients excluded from the study, there was no significant difference in recipient age (51 ± 12 versus 50 ± 14 years; $P=0.39$), recipient race (black race 14% versus 11%; $P=0.45$), donor age (33 ± 13 versus 32 ± 12 ; $P=0.66$), ischemic time (216 ± 50 versus 217 ± 53 minutes; $P=0.89$), rejection rate during first year (35% versus 29%; $P=0.35$), or prevalence of diabetes mellitus during the first posttransplant year (32% versus 28%; $P=0.53$). By design of the study, no patient in the study had significant coronary artery disease defined compared with a prevalence of 7.0% at the first annual evaluation in the sample not part of the study.

Immunosuppressive Regimen

Induction therapy was used in all patients and consisted of daclizumab, an anti-interleukin-2 monoclonal antibody, or OKT3, a mouse antibody directed against the CD3 antigen that is closely associated with the T-cell receptor (5 patients). Maintenance immunosuppression consisted of a calcineurin inhibitor (cyclosporine or tacrolimus) and either mycophenolate mofetil or sirolimus. Corticosteroid therapy (methylprednisone) was initiated immediately postoperatively and progressively tapered over 1-year posttransplant in the absence of rejection. Cytomegalovirus prophylaxis consisted of valganciclovir for a total of 6- to 12-month duration of prophylaxis in patients with evidence of seropositive donor or recipient status. Intravenous cytomegalovirus immunoglobulin therapy was given in seronegative recipients of seropositive donors.

Echocardiography

Digitized echocardiographic studies were analyzed by a reader (F.H.) blinded to the hemodynamic data using quantitative criteria in accordance with the published guidelines of the American Society of Echocardiography.¹⁹ Echocardiographic studies were obtained within 24 hours of invasive measurements for each patient. Left ventricular internal dimension in diastole, as well as left ventricular septal wall thickness and left ventricular posterior wall thickness in diastole, was measured in the parasternal long-axis view using a 2-dimensional

echocardiographic method and averaged over 3 measures. In measuring the septal wall thickness, careful attention was taken to exclude the right ventricular septal bands; similarly, careful attention was taken not to include chordae in the measurement of the posterior wall.

Small left ventricular dimension was defined as left ventricular internal dimension (LVID) <2.4 cm/ m^2 for women and <2.2 cm/ m^2 for men according to the criteria established by the American Society of Echocardiography.¹⁹ Concentric ventricular remodeling was identified in the presence of a relative wall thickness (2 left ventricular posterior wall thickness in diastole/left ventricular internal dimension in diastole) ≥ 0.42 .¹⁹ Left ventricular hypertrophy was defined by a ventricular mass >96 g/ m^2 for women or >116 g/ m^2 for men according to the threshold values established by the American Society of Echocardiography.¹⁹ LVEF was calculated using the biplane Simpson method of disk. Myocardial performance indices were calculated, as previously described, as the ratio of isovolumic relaxation and contraction times divided by the ejection time.²⁰ To measure the myocardial performance index, all time intervals were averaged over 3 consecutive cycles. Myocardial performance index represents a measure of both systolic and diastolic function.²¹ A higher value of the myocardial performance index is associated with worse ventricular function.^{20,21} Normal values of myocardial performance indices have been previously established; for the right ventricle, normal values of right ventricular myocardial performance index (RVMPI) are 0.28 ± 0.04 , and for the left ventricle, normal values of left ventricular myocardial performance index have been previously reported to be 0.38 ± 0.05 .^{20,21} Tissue Doppler parameters were not available for data analysis.

Coronary Physiology Measurements

Microcirculatory disease was quantified using the IMR (Figure 1). To measure IMR, a 0.014-inch coronary pressure wire (Radi Medical Systems) was calibrated outside of the body and then advanced through a 6F guiding catheter to position the pressure sensor at the ostium of the guiding catheter where equal pressure readings by the guiding catheter and the pressure wire were confirmed.^{3,10} The wire was then advanced in the distal portion of the left anterior descending artery. The shaft of the pressure wire acts as a proximal thermistor, and the pressure sensor acts as a distal thermistor. Room temperature saline was injected down the left anterior descending artery in 3-mL aliquots 3 \times , and the resting mean transit time of the saline was recorded and averaged. Maximal hyperemia was then induced by administration of intravenous adenosine (140 μ g/kg per min) via a central venous line, and the hyperemic mean transit time was determined by averaging the transit times after 3 injections of 3 mL of saline. The IMR was calculated by dividing pressure by flow—in this case, the distal pressure by the inverse of the hyperemic mean transit time or, more simply, distal pressure multiplied by the hyperemic mean transit time. A threshold value of 20 mmHg seconds for IMR was used to define microvascular dysfunction based on prior work by our group in heart transplantation.^{3,13} The threshold value corresponded to the median value of patients without a history of hemodynamically compromising rejection and defines patients with lower versus higher microvascular dysfunction. In a subgroup of 28 patients (44%), microvascular function was also available at baseline. CFR by thermidilution was calculated by dividing the resting mean transit time by the hyperemic mean transit time (Figure 1). A CFR threshold value <2.5 was considered abnormal based on previously published physiological and outcome studies.^{14,22}

Epicardial physiology was measured using FFR. FFR was measured by dividing the mean distal pressure by the mean aortic pressure during maximal hyperemia. Significant epicardial CAV was defined by a luminal stenosis of $>50\%$ by quantitative angiography in the left main or primary vessel.¹⁸

Clinical Definitions and Combined Outcome

Donor-recipient mismatch was defined clinically as a 20% weight difference between the recipient and the donor at the time of transplant. Diabetes mellitus was defined according to the American Diabetes Association criteria as a fasting glucose >7 mmol/L (126 mg/dL) requiring at least 3 months of hypoglycemic or insulin therapy.²³

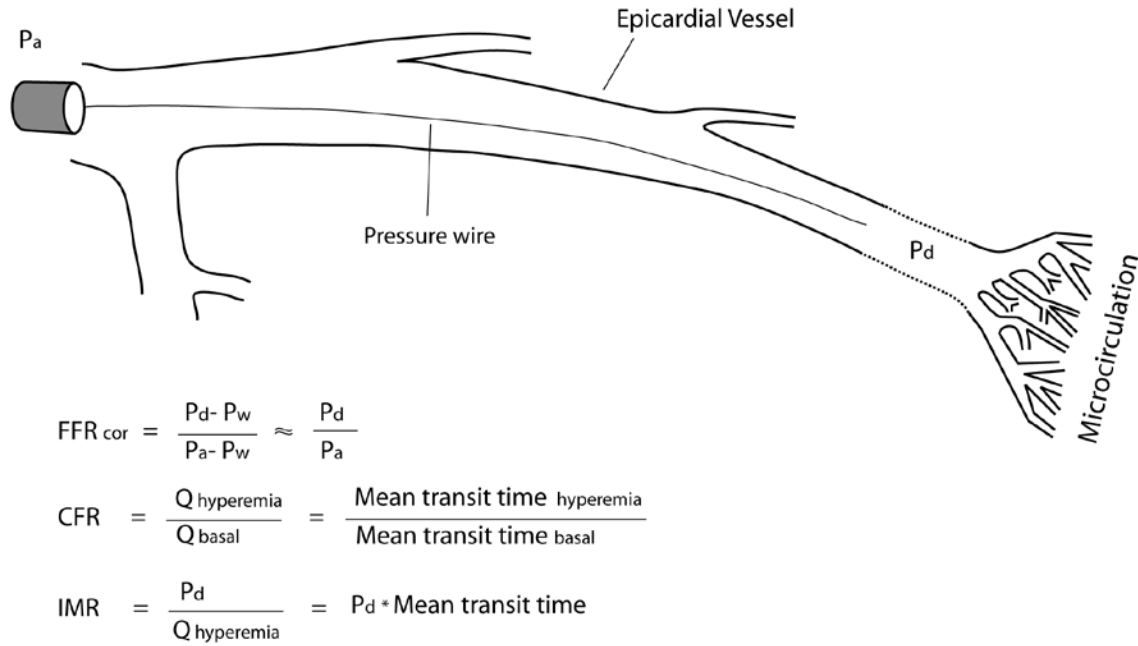


Figure 1. Common measures of coronary physiology in clinical practice. Epicardial coronary physiology is usually estimated using fractional flow reserve (FFR) and is calculated during maximal hyperemia conditions as the ratio of distal pressure (Pd) to mean aortic pressure (Pa). Coronary flow reserve (CFR) assesses both the epicardial and microvasculature physiology and is calculated as the ratio of hyperemic to basal flow, which can be simplified as the ratio of basal to hyperemic mean transit time. Index of microcirculatory resistance (IMR) assesses more specifically the microcirculation and is calculated as the product of Pd and mean transit time. Pw indicates coronary wedge pressure.

Glomerular filtration rate was estimated using the Modification of Diet in Renal Disease Study equation.²⁴

For the purposes of an exploratory analysis, patients were followed for up to 5 years for the combined outcome of death, graft failure, or significant allograft vasculopathy. Graft failure was diagnosed if patients met the Framingham Criteria for Congestive Heart Failure and had evidence of new-onset systolic dysfunction with LVEF <45% and at least 25% relative change from baseline.¹⁷ Significant epicardial CAV was defined by a luminal stenosis of >50% by quantitative angiography by a reader blinded to the other clinical variables.

Statistical Methods

Results are expressed as mean±SD for continuous variables or as number of cases and percentage for categorical variables. Comparison of groups was performed using Student *t* test for continuous variables and χ^2 test or Fisher exact Test, as appropriate, for categorical variables. For the χ^2 test, the *P* value reported corresponds to the Pearson χ^2 without continuity correction. Logistic regression analysis was used to determine the factors independently associated with microvascular dysfunction at 1 year. We used a stepwise regression analysis combining forward selection and backward elimination; variables with *P*<0.30 were entered in the regression and variables with *P*>0.5 were removed from the model. In the subgroup of 29 patients who had both baseline and 1-year values of microvascular function, comparison of baseline and 1-year values of IMR, cardiac index, pulmonary capillary wedge pressure (PCWP), and right atrial pressure (RAP) was made using paired *t* test. Logistic regression analysis was used to determine the factors independently associated with change in cardiac index, PCWP, or RAP from baseline to 1 year. For the purpose of an exploratory outcome analysis, Cox proportional hazard analysis was performed to determine the hazard ratio of factors associated with the combined outcome of death, graft failure, or epicardial allograft vasculopathy. Because of the small number of events, multivariable survival analysis was not performed. Kaplan-Meier survival curve was used to represent the survival of patients with or without microvascular dysfunction. *P*<0.05 was considered to be statistically significant. Statistical analysis was performed using the PASW software (PASW 18.0 Inc, Chicago, IL).

Results

Patient Characteristics

The mean age at transplant was 51±12 years, and the majority of patients (79%) were men (Table 1). Eighteen patients (29%) were transplanted for ischemic cardiomyopathy, donor age was 33±13 years, and mean ischemic time was 216±50 minutes. Donor-recipient size mismatch defined by >20% weight difference between donor and recipient (donor less than recipient) was present in 12 patients (19%). All patients were treated with triple immunosuppressive therapy (prednisone, cyclosporine, or tacrolimus and mycophenolate mofetil or sirolimus), and the majority received statin therapy (96%). The mean cyclosporine level at the first annual evaluation was 156±61 ng/mL (n=44), and the mean tacrolimus level was 7.5±4.1 ng/mL (n=19).

Rejection during the first posttransplant year was documented in 22 patients (35%), 7 (32%) of whom had evidence of associated systolic graft dysfunction at the time of rejection (relative change in LVEF >25% from baseline). Of the 7 patients with rejection and hemodynamic compromise, 3 were diagnosed with noncellular rejection (International Society for Heart and Lung Transplantation grade <2R).

At 1-year posttransplant, the average LVID in diastole was 4.6±0.4 cm and 2.3±0.3 cm/m² when indexed to body surface area, the relative wall thickness was 0.43±0.06, and the indexed left ventricular (LV) mass was 82±18 g/m². Small indexed LV dimension based on the American Society of Echocardiography criteria was observed in 36 patients (57%). The majority of patients with weight mismatch had smaller donor hearts based on indexed LVID at 1 year (75%), but 48% of patients without weight mismatch also had small indexed LV size at 1 year.

Table 1. Comparison of Factors Potentially Associated With Early Microvascular Dysfunction

Characteristics	Sample (n=63)	IMR >20 (n=29)	IMR ≤20 (n=34)	P
Demographic factors				
Age, y	51±12	51±12	51±12	0.84
Female sex	13 (21%)	4 (14%)	9 (26%)	0.35
Black race	9 (14%)	4 (14%)	5 (15%)	1.0
Donor characteristics				
Donor age, y	33±13	32±14	34±13	0.55
Ischemic time, min	216±50	223±45	210±9	0.32
Donor-recipient mismatch, wt	14 (2%)	6 (21%)	8 (23%)	0.97
Cytomegalovirus mismatch	18 (29%)	7 (24%)	11(32%)	0.47
Pretransplant PRA >10%	4 (6%)	1 (3%)	3 (9%)	0.62
Ventricular size and remodeling				
Small indexed LV size	35 (56%)	21 (72%)	14 (41%)	0.01
Concentric remodeling	28 (44%)	15 (52%)	13 (34%)	0.28
LV mass index	82±18	82±18	81±18	0.93
LVH	7 (11%)	3 (10%)	4 (12%)	0.86
History of rejection in the first year	22 (35%)	15 (52%)	7 (21%)	0.01
Comorbid conditions				
Obesity (BMI >30)	28 (40%)	14 (48%)	14 (41%)	0.57
Diabetes mellitus	20 (32%)	9 (31%)	11 (32%)	1.0
Systemic hypertension	11 (17%)	6 (21%)	5 (15%)	0.74
Laboratory values				
GFR, mL/min per 1.73 m ²	66±27	63±30	68±24	0.50
Drug levels				
Cyclosporine, ng/mL, n=44	152±74	157±82	147±72	0.69
Tacrolimus, ng/mL, n=19	6.8±4.3	6.4±4.4	7.2±4.3	0.70
Treatment regimen				
Sirolimus-based regimen	16 (25%)	5 (17%)	11(32%)	0.24
Coronary physiology				
Fractional flow reserve	0.86±0.06	0.87±0.07	0.85±0.06	0.37

IMR indicates index of microcirculatory resistance; PRA, panel reactive antibody; LV, left ventricular; LVH, left ventricular hypertrophy; BMI, body mass index; GFR, glomerular filtration rate.

*Defined according to the American Society of Echocardiography guidelines for small LV size.

Concentric LV remodeling defined by a relative wall thickness >0.42 was seen in 28 patients (44%), whereas LV hypertrophy by indexed mass criteria was present in 7 patients (11%). LVEF was 61±8%, and all patients had an LVEF >45% at the time of their annual evaluation. Left ventricular myocardial performance index was 0.46±0.08 (reference, 0.39±0.05), and RVMPI was 0.39±0.13 (reference, 0.28±0.04). On right heart catheterization, systolic blood pressure was 123±16 mmHg, mean RAP was 6±5 mmHg, mean pulmonary arterial pressure (mPAP) was 21±8 mmHg, mean PCWP was 11±5 mm, Hg and cardiac index was 3.4±0.8 L/min per m².

Coronary Physiology Measures at 1 Year

The average FFR was 0.86±0.06 (median, 0.87), the average IMR was 23±17 (median, 19), and the average CFR was

3.4±1.9 (median, 2.9). Four patients in the study had FFR values <0.75 in the absence of severe focal stenosis, suggestive of diffuse disease (Figure 2).

Microvascular dysfunction, predefined by an IMR>20, was observed in 29 patients at 1 year (46%). There was no significant difference between FFR of patients with and without microvascular dysfunction (0.87±0.07 versus 0.85±0.06; *P*=0.87). When using a CFR threshold of 2.5 to classify microvascular dysfunction, there was a concordance rate of 75% between microvascular dysfunction defined by CFR or IMR (Figure 2).

Factors Associated With Early Microvascular Dysfunction

A higher proportion of patients with microvascular dysfunction had a history of acute rejection during the first post-transplant year (*P*=0.028). Smaller LV ventricles based on indexed Left ventricular internal dimension in diastole were also more common among patients with microvascular dysfunction (*P*=0.20) (Table 1). There was a trend for an association between sirolimus-based therapy and a lower incidence of microvascular dysfunction (32% versus 17%; *P*=0.17). There was no significant difference in body mass index, obesity (body mass index >30), prevalence of diabetes mellitus, cyclosporine drug levels, concentric left ventricular remodeling, or left ventricular hypertrophy between patients with or without microvascular dysfunction (Table 1). On logistic multivariable analysis that included covariates with *P*<0.3, both acute rejection and smaller left ventricular size were independently associated with microvascular dysfunction at 1 year (Table 2).

Functional Correlates of Microvascular Dysfunction

Patients with microvascular dysfunction (IMR >20) had lower cardiac index and higher values of myocardial performances indices, suggestive of impaired ventricular function (Table 3 and Figure 3). There was, however, no significant differences between groups for PCWP, RAP, LVEF, or right ventricular fractional area change.

On multivariable linear regression analysis, microvascular dysfunction (IMR >20) was an independent covariate for cardiac index, as well as RVMPI and left ventricular myocardial performance index, but not LVEF. The covariates considered in the model were based on both statistical and clinical significance and included IMR >20, rejection history, diabetes mellitus, donor age, heart rate, and systolic blood pressure. For cardiac index, IMR >20 was the only independent variable in the regression equation, with a *P*=0.03 and a coefficient of determination *R*²=0.16. For left ventricular myocardial performance index, IMR >20 was the only independent variable in the regression equation, with a *P*<0.01 and a coefficient of determination *R*²=0.29. For RVMPI, IMR >20 was an independent variable in the regression equation with a *P*<0.01, whereas history of rejection in the first year had a *P*=0.086 and a coefficient of determination *R*²=0.37. For LVEF, no variable was retained in the regression equation.

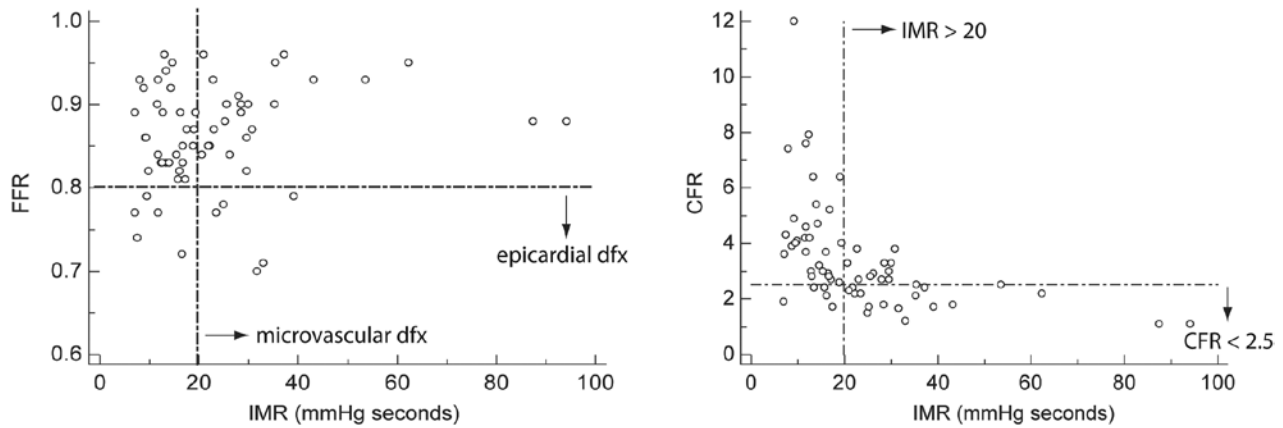


Figure 2. Relationship between index of microcirculatory resistance (IMR) and fractional flow reserve (FFR) (**left panel**) and IMR and coronary flow reserve (CFR) (**right panel**). The **left panel** shows that in the majority of patients, microvascular dysfunction occurs in patients with FFR >0.75. The **right panel** emphasizes the relationship between IMR and CFR using usual threshold to microvascular dysfunction.

Change in Microvascular Dysfunction at Baseline and 1 Year and Its Relationship to Ventricular Function

In 28 patients, microvascular function and hemodynamics were available at both baseline and 1 year. Baseline studies were performed on average at 4 weeks after transplant. Patients at baseline had a lower hemoglobin level (102±12 versus 118±16 g/L; *P*<0.001) and a lower heart rate (79±11 versus 85±11 beats per minute; *P*=0.038). The average IMR decreased during the first posttransplant year, with some patients showing greater change in microvascular function than others (27±15 versus 19±8; *P*=0.01) (Figure 4). The average cardiac index was 3.5±0.5 L/min per m² at baseline and 3.3±0.9 L/min per m² at 1 year (*P*=0.36) (Figure 4). The average stroke volume index was 45±9 mL/m² at baseline and 40±12 mL/m² at 1 year (*P*=0.059). The average PCWP was 13±7 mm Hg at baseline and 12±6 mm Hg at 1 year (*P*=0.23).

When analyzing the relationship between the dynamic change in IMR and cardiac index, stroke volume index, PCWP, and RAP, a significant correlation was found between change in IMR and change in cardiac index (*r*=−0.57; *P*< 0.001) or change in stroke volume index (*r*=−0.57; *P*=0.001) (Figure 4, lower panel). No relationship was found between change in IMR and change in PCWP (*P*=0.97) or change in IMR and change in RAP (*P*=0.74). To determine whether a change in cardiac index or stroke volume was independently associated with a change in IMR, we conducted a multivariable model with a change in microvascular function, history of rejection, history of hemodynamically compromising rejection, diabetes

mellitus, donor age, and relative change in hemoglobin as the potential independent variables. We found that a change in cardiac index was independently associated with a change in IMR (*P*=0.001) and a history of hemodynamically compromising rejection (*P*=0.032) with an *R*² of 0.43. Similarly, a change in stroke volume index was also associated with a change in IMR (*P*=0.003) and a history of hemodynamically compromising rejection (*P*=0.014) with an *R*² of 0.44.

Exploratory Outcome Analysis

Patients were followed for up to 5 year after heart transplantation for outcome analysis. Because of the small sample size, our outcome analysis was only intended to be exploratory. The mean follow-up time was 3.5±0.5 years, and the combined end

Table 2. Unadjusted and Multivariable Correlates of Microvascular Dysfunction (IMR >20)

	Unadjusted Correlates		Multivariable Correlates	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
Acute rejection first year	4.13 (1.37–12.48)	0.011	4.23 (1.33–13.41)	0.015
LV size mismatch	3.17 (1.12–9.00)	0.029	3.25 (1.07–9.84)	0.036
Sirolimus therapy	0.44 (0.13–1.45)	0.17

IMR indicates index of microcirculatory resistance; OR, odds ratio; LV, left ventricular.

Table 3. Functional Characteristics Associated With Microvascular Dysfunction

Characteristics	IMR >20 (n=29)	IMR ≤20 (n=34)	<i>P</i>
Right heart catheterization			
Heart rate, bpm	92±11	87±11	0.11
SBP, mmHg	122±16	124±15	0.51
RAP, mmHg	7±6	5±3	0.13
mPAP, mmHg	21±6	21±10	0.93
PCWP, mmHg	12±5	10±5	0.33
CI, L/min per m ²	3.1±0.7	3.5±0.7	0.02
PVRI, WU m ²	3.0±1.4	2.9±2.0	0.84
Echocardiography			
LVEF, %	60±8	62±9	0.47
RVFAC, %	40±9	44±8	0.12
LVMPI	0.54±0.09	0.43±0.09	<0.01
RVMPI	0.47±0.14	0.32±0.05	<0.01

IMR indicates index of microcirculatory resistance; bpm, beats per minute; SBP, systolic blood pressure; RAP, right atrial pressure; mPAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; CI, cardiac index; PVRI, pulmonary vascular resistance index; LVEF, left ventricular ejection fraction; RVFAC, right ventricular fractional area change; LVMPI, left ventricular myocardial performance indices; RVMPI, right ventricular myocardial performance indices.

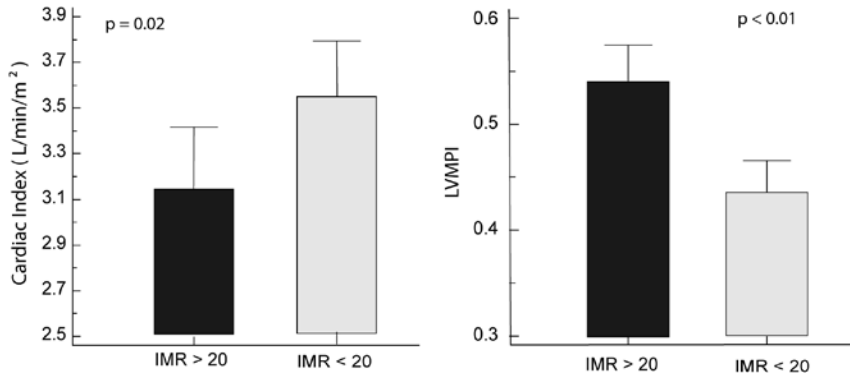


Figure 3. Differences in cardiac index and left ventricular myocardial performance indices (LVMPI) between patients with or without microvascular dysfunction (index of microcirculatory resistance [IMR] >20).

point was reached in 22 patients. Six patients died, 3 as a result of progressive graft failure, 2 as a result of acute graft failure, and 1 secondary to sudden cardiac death. Twelve patients had evidence of symptomatic graft dysfunction for >3 months of duration, and 4 patients had evidence of significant allograft vasculopathy on coronary angiography in the absence of heart failure (1 patient had a luminal stenosis of the left anterior

descending artery of 60% and 3 others had luminal stenosis of >70%, 1 patient undergoing coronary artery stenting). On univariable analysis, IMR >20 was significantly associated with outcome on univariable analysis (Table 4). Figure 5 illustrates the Kaplan-Meier survival curve associated with IMR >20. A history of hemodynamically compromising rejection during the first year was the strongest factor associated with

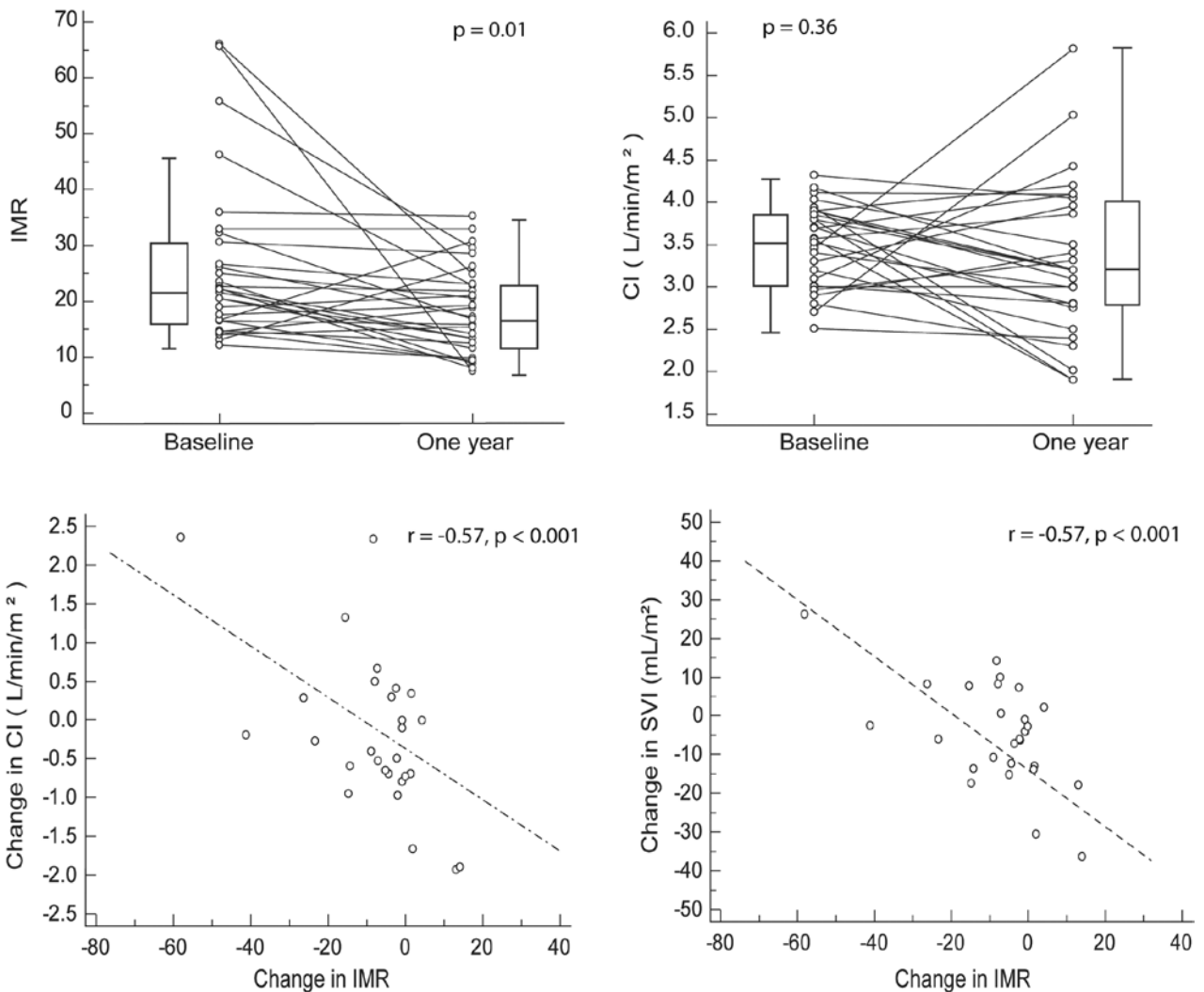


Figure 4. Change in index of microcirculatory resistance (IMR) and cardiac index (CI) from baseline to 1 year. The lower panels show the relationship between change in CI and change in IMR, as well as the relationship between change in indexed stroke volume (SVI) and change in IMR.

Table 4. Exploratory Outcome Analysis of Factors Associated With the Combined Outcome of Death or Graft Failure or Significant Allograft Vasculopathy

	Unadjusted Correlates of Outcome	
	HR (95% CI)	P
Microvascular dysfunction at 1 y (IMR >20)	2.52 (1.04–5.91)	0.04
Microvascular dysfunction at 1 y (CFR <2.5)	1.3 (0.6–3.0)	0.52
Diabetes mellitus	2.4 (1.03–5.6)	0.04
History of rejection during the first year	2.1 (0.9–4.8)	0.09
History of hemodynamically compromising rejection during the first year	12.6 (4.2–38.4)	<0.01
Right atrial pressure per 5 mm Hg	1.8 (1.2–2.8)	<0.01
Pulmonary capillary wedge pressure per 5 mm Hg	1.5 (1.04–2.0)	0.03
Cardiac index per 0.5 L/min per m ² decrease	1.4 (0.99–2.1)	0.06
LVEF per 5% decrease	1.1 (0.9–1.4)	0.39
LVEF <55%	1.7 (0.6–4.7)	0.28
Left ventricular size mismatch	1.6 (0.7–3.7)	0.33
Donor age >40 y	1.7 (0.7–4.0)	0.21

IMR indicates index of microcirculatory resistance; HR, hazard ratio; CFR, coronary flow reserve; LVEF, left ventricular ejection fraction.

the combined end point. Other factors significantly associated with outcome included rejection history, diabetes mellitus, RAP, and PCWP.

Intraobserver Variability in Measurements of Echocardiographic Indices

To determine intraobserver variability, a blinded reader (F.H.) repeated the measurements on 30 studies. There was good concordance between measures of LV and right ventricular parameters. For LVID, the difference in absolute measurement was 0.6±1.3 mm, with an intraclass correlation coefficient of 0.94. For LVEF, the difference in absolute measurement was 1.4±2.6%, with an intraclass correlation coefficient of 0.85. For left ventricular myocardial performance index, the average difference in absolute measurement was 0.02±0.05, with an intraclass correlation coefficient of 0.91. For RVMPI, the average difference in absolute measurement was 0.03±0.04, with an intraclass correlation coefficient of 0.89.

Discussion

Our study is the first to assess the clinical correlates of early microvasculopathy using IMR, a more specific index of microvascular function. We have found that a history of rejection during the first year and smaller indexed LV dimensions were more common among patients with microvascular dysfunction. Early microvascular dysfunction was also associated with mild lower cardiac index, echocardiographic evidence of mild graft dysfunction, and a higher incidence of death, graft failure, or significant CAV on long-term follow-up.

CAV continues to limit the long-term survival of patients with cardiac transplantation.^{2,25} Although CAV may affect both the epicardial vessels and the microvasculature,

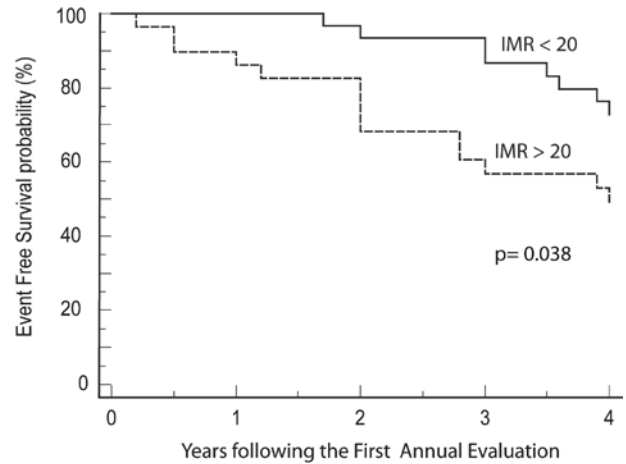


Figure 5. Kaplan-Meier survival curve of patients with evidence of early microvascular dysfunction compared with patients without evidence of microvascular dysfunction. IMR indicates index of microcirculatory resistance.

microvascular dysfunction often occurs in the absence of epicardial disease.^{1,2,22,25} In our study, the majority of patients with evidence of early microvascular dysfunction (86%) had no evidence of impaired epicardial physiology based on FFR, a measure of epicardial physiology. In terms of evolution, microvascular function improves on average from between baseline to 1 year, whereas epicardial physiology measured by FFR worsens as was previously shown in the Physiologic Investigation for Transplant Arteriopathy (PITA) II trial.³

Different methods have been developed to assess the microvasculature, with each method having its own advantages and disadvantages.¹⁴ A pathology-based system for grading microvasculopathy based on light microscopy has been recently proposed by Hiemann et al⁴ based on the histological characteristics of endothelium, wall, and lumen. Stenotic microvasculopathy was defined as a ratio of luminal radius to wall thickness <1. Functional assessment of the microvasculature is clinically based on assessing both endothelial-dependent and endothelial-independent vasodilation.^{4–6,14,22,25,26} Endothelial-dependent vasodilation is usually assessed using acetylcholine, which acts on the endothelium, whereas endothelial-independent assay mainly involves agents that act on vascular smooth muscles cells, usually with adenosine. In terms of indices, although CFR has been the most commonly used index, IMR has the advantage of being more specific for the microvasculature and less dependent on hemodynamics. In our study, we assessed the microvasculature using IMR, an endothelium-independent vasodilation with adenosine. Our study confirms that microvascular dysfunction based on CFR and IMR is often but not always concordant (75% of cases). In fact, several patients with normal microvasculature function based on IMR may have CFR <2.5; similarly, several patients with IMR >20 have CFR >2.5.

Consistent with the study of Osto et al,²⁷ we have found that rejection is more common among patients with microvascular disease. Osto et al²⁷ have recently found that, in the absence of significant epicardial CAV, rejection score was the only independent correlate of microvascular dysfunction defined using

CFR during the first 5 years of posttransplant. Compared with the study of Osto et al,²⁷ we defined microvascular dysfunction invasively using IMR, and every patient was assessed systematically during the first posttransplant year. The association between rejection and microvascular dysfunction underscores the importance of immune mechanisms in CAV.¹ This association could be even stronger with antibody-mediated rejection, which is known to target the endothelium of small vessels.^{28,29} At our center, complement split product C4d staining is not routinely performed on biopsy, and it is difficult for us to specifically study this association.

The association between LVID and microvascular dysfunction is novel and needs to be confirmed in future studies. In our study, our classification of smaller donor size at 1 year was based on the criteria of the American Society of Echocardiography.¹⁹ This definition differs from the usual definition of undersized donor hearts, which was based on a 20% weight difference between donor and recipients but has the advantage of being based on direct measurements of ventricular size.³⁰ Prior studies have shown that undersized donor hearts based on weight differences were associated with a higher likelihood of mortality, especially in patients with increased pulmonary vascular resistance.³⁰ Theoretically, smaller donor hearts could have rarefaction of the microvasculature, which could contribute to increased shear stress.³¹ Previous studies in heart transplantation and systemic hypertension have also shown that left ventricular hypertrophy was associated with microvascular dysfunction.^{27,31}

Functionally, microvascular dysfunction was associated with evidence of mild graft dysfunction based on both cardiac index and right and left myocardial performance index. Myocardial performance index represents an index of global systolic and diastolic function and is measured as the ratio between isovolumic and relaxation time divided by ejection time. In patients with normal microvascular function, both left and right myocardial performance indices were close to the reference range of healthy volunteers.^{20,21} The association between microvascular function and graft function is further supported by the fact that a dynamic change in IMR was an independent correlate of a change in cardiac index between baseline and 1 year. These findings are consistent with the work of Weis et al²⁵ who have shown that the presence of endothelium-independent microvascular dysfunction predicted deterioration of left ventricular systolic function both at rest and during exercise (n=17). Of importance, causal relationship may not be directly inferred from our findings, and future longitudinal studies with larger sample size analyzing the dynamic relationship between microvascular function and development of graft failure are needed.

Our exploratory analysis also suggests the importance of microvascular function. Because of the small sample size, multivariable analysis could not be performed without overfitting the model. This finding is consistent with prior studies that have called attention to the importance of microvascular disease in heart transplantation. Hiemann et al⁴ have recently found that stenotic microvascular disease detected

on endomyocardial biopsy, epicardial coronary disease, and diabetes mellitus was an independent correlate of posttransplant mortality. Earlier pathological studies by Billingham et al³² have also found an association between microvascular disease and sudden cardiac death in heart transplantation. Studies analyzing the clinical correlates of CFR-derived measures of microvascular function have found variable relationships with outcome. In the study by Hollenberg et al,^{5,6} endothelial-dependent microvascular response to acetylcholine assessed by CFR but not endothelial-independent response by adenosine (CFR based) was related to the development of epicardial CAV or cardiac death. In the study by Kübrich et al,⁸ an association between microvascular endothelium-independent dysfunction assessed by CFR and adverse outcome was found on univariable analysis but not on multivariable analysis.

Our study has several clinical implications. First, because of the interrelationship among microvascular function, rejection, and graft function, powering the studies adequately to prove the independent predictive value of microvascular dysfunction will require a large sample size. Second, future studies are necessary to investigate whether early treatment of patients with evidence of microvascular dysfunction but without evidence of early CAV on intravascular ultrasound imaging will improve outcome. A study by Sinha et al³³ showed that sirolimus-based therapy initiated early after transplant was associated with improved coronary artery physiology involving both the epicardial vessel and the microvasculature. Prior studies have shown that treatment with mammalian targets of rapamycin inhibitors, such as sirolimus or everolimus, decreases the progression of CAV.^{34,35} Whether the assessment for early microvascular dysfunction should be made at 6 months or 1 year is also a subject of future research.

This study has several limitations. First, the small sample size limits our ability to conduct multivariable analysis without overfitting our model. Also, the cohort did not represent consecutive patients undergoing heart transplantation. Furthermore, although IMR is a more reproducible marker of microvascular function than CFR, IMR does not take into account all factors involved in microvascular physiology, such as true back pressure, coronary capacitance, or tissue volume supplied by the artery.¹⁴ Furthermore, future studies will also be needed to better understand the relationship between IMR and microvasculopathy detected in biopsy specimens.⁴

Conclusions

Microvascular dysfunction at 1-year posttransplant is associated with lower cardiac index and echocardiographic indices of graft dysfunction. Changes in microvascular function between baseline and 1 year were also associated with changes in cardiac index. History of rejection and smaller donor hearts assessed using echocardiography are key correlates of microvascular dysfunction. Future multicenter studies will be needed to validate these findings and to determine predictive role of early microvascular dysfunction on long-term outcomes after heart transplantation. Furthermore,

future trials designed to determine whether early therapy of microvascular dysfunction would improve long-term outcome are needed.

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Disclosures

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CLINICAL PERSPECTIVE

Cardiac allograft vasculopathy continues to limit the long-term survival of patients with cardiac transplantation. Cardiac allograft vasculopathy may affect both epicardial vessels and the microvasculature. In this study, we sought to determine the prevalence and clinical correlates of early microvascular dysfunction defined using the index of microcirculatory resistance. We found that early microvascular dysfunction occurs in the majority of patients in the absence of functionally significant epicardial disease. Early microvascular dysfunction was more common among patients with a history of rejection and smaller left ventricular size. Microvascular dysfunction at 1 year was also associated with evidence of mild graft dysfunction and a higher likelihood of death, epicardial allograft vasculopathy, or graft failure. Future studies are needed to determine whether early initiation of target of rapamycin inhibitors, such as sirolimus or everolimus, in patients with evidence of microvascular dysfunction will improve long-term outcomes.

Clinical and Functional Correlates of Early Microvascular Dysfunction After Heart Transplantation

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