Clinical and Functional Correlates of Early Microvascular Dysfunction Following Heart Transplantation

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Abstract

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 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 (IMR). The presence of microvascular dysfunction, pre-defined by an IMR > 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 an OR=4.0 (1.3-12.8) and OR=3.6 (1.2-11.1), respectively. Patients with microvascular dysfunction had lower cardiac index (3.1 ±0.7 vs. 3.5 ± 0.7 L/min/m², p=0.02), and mild graft dysfunction measured by echocardiography-derived left and right myocardial performance indices [(0.54 ± 0.09 vs. 0.43 ± 0.09, p< 0.01) and (0.47±0.14 vs. 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 post transplant (hazard ratio of 2.52, 95% confidence interval between 1.04 and 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 IMR at 1 year was also associated with worse graft function and possibly worse clinical outcomes.

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.\textsuperscript{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.\textsuperscript{2} Cardiac allograft vasculopathy is a progressive disorder that may affect both the epicardial vessels and the microcirculation of the heart.\textsuperscript{1-3}

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

At this time, the determinants and functional consequences of early microvascular defined using the IMR have not been established. For this study, we sought to determine whether a history of acute rejection during the first post-transplant 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 according to Stanford Medical Center’s Human Investigation Review Board (IRB) prior to coronary physiology measurements. Patients included in the analysis were enrolled as part of NIH funded trials, 1 K23 HL072808-01A1, 1 PO1-AI50153, and 5 R01 HL093475-02) Data collection was performed by two 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 post-transplant 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. Both cellular (ISHLT grade ≥ 2R) and non-cellular rejection with hemodynamic compromise (decrease in relative left ventricular ejection fraction of greater than 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. 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 pre-
defined by a luminal stenosis of greater than 50% by quantitative angiography. 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). When compared to patients excluded from the study, there was no significant difference in recipient age (51±12 vs. 50±14 years, p=0.39), recipient race (Black race 14% vs. 11%, p=0.45), donor age (33±13 vs. 32±12, p=0.66), ischemic time (216±50 vs. 217±53 min, p=0.89), rejection rate during first year (35% vs. 29%, p=0.35) or prevalence of diabetes mellitus during the first post-transplant year (32% vs. 28%, p=0.53). By design of the study, no patient in the study had significant CAD defined compared to a prevalence of 7.0% at the first annual 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 post-operative and progressively tapered over one year post-transplant in the absence of rejection. Cytomegalovirus prophylaxis consisted of valganciclovir for a total of 6 to 12 months duration of prophylaxis in patients with evidence of seropositive donor or recipientstatus. Intravenous
cytomegalovirus immunoglobulin (CMV-IG) 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. Echocardiographic studies were obtained within 24 hours of invasive measurements for each patient. Left ventricular internal dimension in diastole (LVIDd) as well as septal (LVSWT) and posterior wall thickness (LVPWd) were measured in the parasternal long axis view using a two-dimensional echocardiographic method and averaged over three 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) < 2.4cm/m² for women and < 2.2cm/m² for men according to the criteria established by the American Society of Echocardiography. Concentric ventricular remodeling was identified in the presence of a relative wall thickness (2 LVPWd/LVIDd) ≥ 0.42. Left ventricular hypertrophy was defined by a ventricular mass greater than 96 g/m² for women or greater than 116 g/m² for men according to the threshold values established by the American Society of Echocardiography. Left ventricular ejection fraction was calculated using the biplane Simpson method of disc. Myocardial performance indices were calculated as previously described as the ratio of isovolumic relaxation and contraction times divided by the ejection time. To measure the myocardial performance index, all time intervals were averaged over three consecutive cycles. Myocardial performance index (MPI) represents a measure of both systolic and diastolic...
A higher value of the myocardial performance index is associated with worse ventricular function.\textsuperscript{20, 21} Normal values of myocardial performance indices have been previously established; for the right ventricle, normal values of right ventricular MPI (RVMPI) are 0.28 ± 0.04 and for the left ventricle, normal values for the left ventricular MPI (LVMPI) have been previously reported to be 0.38 ± 0.05.\textsuperscript{20, 21} Tissue Doppler parameters were not available for data analysis.

**Coronary physiology measurements**

Microcirculatory disease was quantified using the index of microcirculatory resistance (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 6-French 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.\textsuperscript{3, 10} The wire was then advanced in the distal portion of the left anterior descending artery (LAD). 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 LAD 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 mcg/kg/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 index of microvascular resistance 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.\textsuperscript{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. Coronary flow reserve (CFR) by thermodilution 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 previous published physiological and outcome studies.\textsuperscript{14,22}

Epicardial physiology was measured using fractional flow reserve (FFR). Fractional flow reserve 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 greater than 50% by quantitative angiography in the left main or primary vessel.\textsuperscript{18}

\textbf{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 (DM) 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.\textsuperscript{23} Glomerular filtration rate was estimated using the Modification of Diet in Renal Disease Study equation.\textsuperscript{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.\textsuperscript{17} Significant epicardial CAV was defined by a luminal stenosis of greater than 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’s t-test for continuous variables and Chi-square test or Fisher Exact Test, as appropriate for categorical variables. For the Chi-square test, the p value reported corresponds to the Pearson's Chi-square 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 values < 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, CI, PCWP and 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. Due to 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. A p value < 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 male (Table 1). Eighteen patients (29%) were transplanted for ischemic cardiomyopathy, donor age was 33 ± 13 years and mean ischemic time was 216 ± 50 min. Donor recipient size mismatch defined by more than 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 annial was 156± 61 ng/mL (n=44) and the mean tacrolimus levels was 7.5 ± 4.1 ng/mL (n=19).

Rejection during the first post-transplant year was documented in 22 patients (35%), seven (32%) of whom had evidence of associated systolic graft dysfunction at the time of rejection (relative change in LVEF > 25% from baseline). Of the seven patients with rejection and hemodynamic compromise, three were diagnosed with non-cellular rejection (ISHLT grade < 2R).

At 1 year post-transplant, the average LVID in diastole was 4.6 ± 0.4 cm and 2.3 ± 0.3 cm/m² when indexed to BSA, the relative wall thickness was 0.43 ± 0.06 and indexed LV mass was 82 ± 18 g/m². Small indexed LV dimension based on the ASE 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. Concentric LV remodeling defined by a relative wall thickness > 0.42 was seen in 28 patients (44%) while LV hypertrophy by indexed mass criteria was present in 7
patients (11%). Left ventricular ejection fraction (LVEF) was 61 ± 8% and all patients had an LVEF greater than 45% at the time of their annual evaluation. Left ventricular myocardial performance index was 0.46 ± 0.08 (reference: 0.39 ± 0.05) and RV MPI was 0.39 ± 0.13 (reference: 0.28 ± 0.04). On right heart catheterization, systolic blood pressure was 123± 16 mmHg, mean right atrial pressure was 6±5 mmHg, mean pulmonary arterial pressure (mPAP) was 21 ± 8 mmHg, mean pulmonary capillary wedge pressure (PCWP) was 11 ± 5 mmHg and cardiac index was 3.4 ± 0.8 L/min/m².

**Coronary physiology measures at 1 year**

The average FFR was 0.86 ± 0.06 (median of 0.87), the average IMR was 23 ± 17 (median 19) and the average CFR was 3.4 ± 1.9 (median of 2.9). Four patients in the study had FFR values below 0.75 in the absence of severe focal stenosis suggestive diffuse disease (Figure 2).

Microvascular dysfunction, pre-defined 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 vs. 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 LVIDd was 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% vs. 17%, p = 0.17). There was no significant difference in BMI, obesity (BMI > 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 which included covariates with p values < 0.3, both acute rejection and smaller left ventricular size were independent 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 RVFAC.

On multivariable linear regression analysis, microvascular dysfunction (IMR>20) was an independent covariate for cardiac index as well as RVMPI and LVMPI 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 CI, IMR >20 was the only independent variable in the regression equation with a p= 0.03 and a coefficient of determination R² =0.16. For LVMPI, IMR >20 was the only independent variable in the regression equation with a p < 0.01 and a coefficient of determination R² =0.29. For RVMPI, IMR >20 was an independent variable in the regression equation with a p < 0.01 while history of rejection in the first year had a p= 0.086 and a coefficient of determination R² =0.37. For LVEF, no variable was retained in the regression equation.
Change in microvascular 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 post-transplant. Patients at baseline had a lower hemoglobin level (102 ± 12 vs. 118 ± 16 g/L, p < 0.001) and a lower heart rate (79 ± 11 vs. 85 ± 11 bpm, p=0.038). The average IMR decreased during the first post-transplant year with some patients showing greater change in microvascular function than others (27±15 vs. 19±8, p=0.01) [Figure 4]. The average cardiac index was 3.5 ± 0.5 L/min/m² at baseline and 3.3 ± 0.9 L/min/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 mmHg at baseline and 12±6 mmHg at 1 year (p=0.23).

When analyzing the relationship between the dynamic change in IMR and CI, stroke volume index, PCWP and RAP, a significant correlation was found between change in IMR and change in CI (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 were 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 R² 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 R² of 0.44.
Exploratory outcome analysis

Patients were followed for up to 5 year after heart transplantation for outcome analysis. Due to the small sample size, our outcome analysis was only intended to be exploratory. The mean follow-up time was $3.5 \pm 0.5$ years and the combined end-point was reached in 22 patients. Six patients died, three as a result of progressive graft failure, two as a result of acute graft failure and one secondary to sudden cardiac death. Twelve patients had evidence of symptomatic graft dysfunction for more than 3 months duration and four 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 more than 70%, one 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 the combined end-point Other factor significantly associated with outcome included rejection history, diabetes mellitus, right atrial pressure and pulmonary capillary wedge pressure.

Intra-observer variability in measurements of echocardiographic indices

To determine intra-observer variability, a blinded reader (FH) repeated the measurements on 30 studies. There was good concordance between measures of LV and RV parameters. For LVID, the difference in absolute measurement was $0.6 \text{ mm} \pm 1.3 \text{ mm}$ with an intra-class correlation coefficient (ICC) of 0.94. For LVEF, difference in absolute measurement was $1.4 \pm 2.6 \%$ with an intra-class correlation coefficient (ICC) of 0.85. For LVMI, the average difference in
absolute measurement was $0.02 \pm 0.05$ with an ICC of 0.91. For RVMPI, the average difference in absolute measurement was $0.03 \pm 0.04$ with an ICC 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 mildly 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.

Cardiac allograft vasculopathy continues to limit the long term survival of patients with cardiac transplantation. Although, CAV may affect both the epicardial vessels and the microvasculature, microvascular dysfunction often occurs in the absence of epicardial disease. 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 while epicardial physiology measured by FFR worsens as was previously shown in the PITA II trial.

Different methods have been developed to assess the microvasculature with each method having its own advantages and disadvantages. A pathology based system for grading microvasculopathy based on light microscopy has been recently proposed by Hiemann et al. 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 or independent vasodilation. Endothelial dependent vasodilatation is usually assessed using acetylcholine, which acts on the endothelium while endothelial independent assay mainly involve agents that act on vascular smooth muscles cells, usually with adenosine. In terms of indices, while coronary flow reserve 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 vasodilatation with adenosine. Our study confirms that although microvascular dysfunction based on CFR and IMR are often but not perfectly 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., we have found that rejection is more common among patients with microvascular disease. Osto et al. 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 post transplant. Compared to the study of Osto et al., we defined microvascular dysfunction using invasively using IMR and every patient was accessed systematically during the first post-transplant year. The association between rejection and microvascular dysfunction underscores the importance immune mechanisms in CAV. This association could be even stronger with antibody mediated rejection which is known to target the endothelium of small vessels. At our center C4d staining is not routinely done 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 one year was
based on the criteria of the American Society of Echocardiography. This definition differs from the usual definition of undersized donor hearts which based on a 20% weight difference between donor and recipients but could has the advantage of being based on direct measurements of ventricular size.\textsuperscript{30} Prior studies have shown that undersized donor hearts based on weight differences was associated with a higher likelihood of mortality especially in patients with increased pulmonary vascular resistance.\textsuperscript{30} Theoretically, smaller donor hearts could have rarefaction of the microvasculature which could contribute to increased shear stress.\textsuperscript{31} Previous studies in heart transplantation and systemic hypertension have also shown that left ventricular hypertrophy was associated with microvascular dysfunction.\textsuperscript{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.\textsuperscript{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 al. 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).\textsuperscript{25} 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 have called attention to the importance of microvascular disease in heart transplantation. Hiemann et al. have recently found that stenotic microvascular disease detected on endomyocardial biopsy, epicardial coronary disease and diabetes mellitus were independent correlate of post-transplant mortality.4 Earlier pathological studies by Billingham and colleagues have also found an association between microvascular disease and sudden cardiac death in heart transplantation.32 Studies analyzing the clinical correlates of CFR derived measures of microvascular function have found variable relationships with outcome. In the study of Hollenberg et al., endothelial dependent microvascular response to acetylcholine assessed by CER but not endothelial independent by adenosine (CFR based) was related to development of epicardial CAV or cardiac death.5,6 In the study of Kubrich et al., an association between microvascular endothelium-independent dysfunction assessed by CFR and adverse outcome was found on unvariable analysis but not on multivariable analysis.8

Our study has several clinical implications. First, due to the interrelationship between 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 from Sinha et al. showed that sirolimus based therapy initiated early post transplant was associated with improved coronary artery physiology involving both the epicardial vessel and the microvasculature.33 Prior studies have shown that treatment with mammalian targets of rapamycin inhibitors such as sirolimus or everolimus
decrease 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 Also, future studies will also be needed to better understand the relationship between IMR and microvasculopathy detected in biopsy specimens.4

Conclusion

Microvascular dysfunction at one year post transplant is associated with lower cardiac index and echocardiographic indices of graft dysfunction. Changes in microvascular function between baseline and 1 year was 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 following 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

None.

References


Table 1. Comparison of factors potentially associated with early microvascular dysfunction

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<td>Cytomegalovirus mismatch</td>
<td>18 (29%)</td>
<td>7 (24%)</td>
<td>11 (32%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Pre-transplant PRA&gt; 10%</td>
<td>4 (6%)</td>
<td>1 (3%)</td>
<td>3 (9%)</td>
<td>0.62</td>
</tr>
<tr>
<td><strong>Ventricular size and Remodeling</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small indexed LV size</td>
<td>35 (56%)</td>
<td>21 (72%)</td>
<td>14 (41%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Concentric remodeling</td>
<td>28 (44%)</td>
<td>15 (52%)</td>
<td>13 (34%)</td>
<td>0.28</td>
</tr>
<tr>
<td>LV mass index</td>
<td>82 ± 18</td>
<td>82 ± 18</td>
<td>81 ± 18</td>
<td>0.93</td>
</tr>
<tr>
<td>LVH</td>
<td>7 (11%)</td>
<td>3 (10%)</td>
<td>4 (12%)</td>
<td>0.86</td>
</tr>
<tr>
<td>History of Rejection in the first year</td>
<td>22 (35%)</td>
<td>15 (52%)</td>
<td>7 (21%)</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Comorbid Conditions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesity (BMI &gt;30)</td>
<td>28 (40%)</td>
<td>14 (48%)</td>
<td>14 (41%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>20 (32%)</td>
<td>9 (31%)</td>
<td>11 (32%)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>11 (17%)</td>
<td>6 (21%)</td>
<td>5 (15%)</td>
<td>0.74</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>----------</td>
<td>---------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td><strong>Systemic hypertension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Laboratory values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR (mL/min/1.73m²)</td>
<td>66 ± 27</td>
<td>63 ± 30</td>
<td>68 ± 24</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Drug levels</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporine (ng/mL) n=44</td>
<td>152 ± 74</td>
<td>157 ± 82</td>
<td>147 ± 72</td>
<td>0.69</td>
</tr>
<tr>
<td>Tacrolimus (ng/mL) n=19</td>
<td>6.8 ± 4.3</td>
<td>6.4 ± 4.4</td>
<td>7.2 ± 4.3</td>
<td>0.70</td>
</tr>
<tr>
<td><strong>Treatment regimen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sirolimus based regimen</td>
<td>16 (25%)</td>
<td>5 (17%)</td>
<td>11 (32%)</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Coronary physiology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fractional Flow Reserve</td>
<td>0.86 ± 0.06</td>
<td>0.87 ± 0.07</td>
<td>0.85 ± 0.06</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*Defined according to the ASE guidelines for small LV size. BMI indicates body mass index.
Table 2. Unadjusted and Multivariable Correlates of Microvascular dysfunction (IMR > 20)

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted correlates</th>
<th>Multivariable correlates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Acute rejection first year</td>
<td>4.13 (1.37-12.48)</td>
<td>0.011</td>
</tr>
<tr>
<td>LV size mismatch</td>
<td>3.17 (1.12-9.00)</td>
<td>0.029</td>
</tr>
<tr>
<td>Sirolimus therapy</td>
<td>0.44 (0.13-1.45)</td>
<td>0.17</td>
</tr>
</tbody>
</table>
**Table 3.** Functional Characteristics associated with microvascular dysfunction

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IMR &gt; 20 (n=29)</th>
<th>IMR ≤ 20 (n=34)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right heart catheterization</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>92 ± 11</td>
<td>87 ± 11</td>
<td>0.11</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122 ± 16</td>
<td>124 ± 15</td>
<td>0.51</td>
</tr>
<tr>
<td>RAP (mmHg)</td>
<td>7 ± 6</td>
<td>5 ± 3</td>
<td>0.13</td>
</tr>
<tr>
<td>MPAP (mmHg)</td>
<td>21 ± 6</td>
<td>21 ± 10</td>
<td>0.93</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>12 ± 5</td>
<td>10 ± 5</td>
<td>0.33</td>
</tr>
<tr>
<td>CI (L/min/m²)</td>
<td>3.1 ± 0.7</td>
<td>3.5 ± 0.7</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Echocardiography</strong></td>
<td></td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>60 ± 8</td>
<td>62 ± 9</td>
<td>0.47</td>
</tr>
<tr>
<td>RVFAC (%)</td>
<td>40 ± 9</td>
<td>44 ± 8</td>
<td>0.12</td>
</tr>
<tr>
<td>LV MPI</td>
<td>0.54 ± 0.09</td>
<td>0.43 ± 0.09</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>RV MPI</td>
<td>0.47 ± 0.14</td>
<td>0.32 ± 0.05</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

LVEF indicates left ventricular ejection fraction; RVFAC, right ventricular fractional area change; LVMPI, left ventricular myocardial performance indices; RVMPI, right ventricular myocardial performance indices; SBP, systolic blood pressure; RAP, right atrial pressure; MPAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; CI, cardiac index and PVRI, pulmonary vascular resistance index.
**Table 4.** Exploratory outcome analysis of factors associated with the combined outcome of death or graft failure or significant allograft vasculopathy

<table>
<thead>
<tr>
<th>Unadjusted correlates of outcome</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microvascular dysfunction at 1 year (IMR &gt; 20)</td>
<td>2.52 (1.04 to 5.91)</td>
<td>0.04</td>
</tr>
<tr>
<td>Microvascular dysfunction at 1 year (CFR &lt; 2.5)</td>
<td>1.3 (0.6 to 3.0)</td>
<td>0.52</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.4 (1.03 to 5.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>History of rejection during the first year</td>
<td>2.1 (0.9 to 4.8)</td>
<td>0.09</td>
</tr>
<tr>
<td>History of hemodynamically compromising rejection during the first year</td>
<td>12.6 (4.2 to 38.4)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Right atrial pressure per 5 mmHg</td>
<td>1.8 (1.2 to 2.8)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure per 5 mmHg</td>
<td>1.5 (1.04 to 2.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Cardiac index per 0.5 L/min/m² decrease</td>
<td>1.4 (0.99 to 2.1)</td>
<td>0.06</td>
</tr>
<tr>
<td>LVEF per 5% decrease</td>
<td>1.1 (0.9 to 1.4)</td>
<td>0.39</td>
</tr>
<tr>
<td>LVEF lower than 55%</td>
<td>1.7 (0.6 to 4.7)</td>
<td>0.28</td>
</tr>
<tr>
<td>Left ventricular size mismatch</td>
<td>1.6 (0.7 to 3.7)</td>
<td>0.33</td>
</tr>
<tr>
<td>Donor age older than 40 year old</td>
<td>1.7 (0.7 to 4.0)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

LVEF indicated left ventricular ejection fraction.
Figure Legends

**Figure 1.** Common measures of coronary physiology in clinical practice. Epicardial coronary physiology is usually estimated using 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 microvascular 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. IMR assesses more specifically the microcirculation and is calculated as the product of Pd and mean transit time. Pw represents coronary wedge pressure.

**Figure 2.** Relationship between IMR and FFR (left panel) and IMR and 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.

**Figure 3.** Differences in cardiac index and LV MPI between patients with or without microvascular dysfunction (IMR> 20).

**Figure 4.** Change in IMR and cardiac index from baseline to one 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 and change in IMR.

**Figure 5.** Kaplan-Meier survival curve of patients with evidence of early microvascular dysfunction compared to patients without evidence of microvascular dysfunction.
FFR_{cor} = \frac{P_d - P_w}{P_a - P_w} \approx \frac{P_d}{P_a}

CFR = \frac{Q_{hyperemia}}{Q_{basal}} = \frac{\text{Mean transit time }_{hyperemia}}{\text{Mean transit time }_{basal}}

IMR = \frac{P_d}{Q_{hyperemia}} = P_d \times \text{Mean transit time}
Clinical and Functional Correlates of Early Microvascular Dysfunction Following Heart Transplantation

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