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Hemodynamic Response to Acute Volume Load and Endomyocardial NO-synthase Gene Expression in Heart Transplant Recipients

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Background. A pulmonary capillary wedge pressure (PCWP) >18 mm Hg following volume load has been proposed as a partition value for the detection of heart failure with preserved ejection fraction. As hemodynamic changes in filling pressures (FP) have been attributed to a nitric oxide (NO)-mediated rightward shift of the pressure-volume relationship, we investigated the hemodynamic response to volume load in heart transplant recipients (HTx) and examined the role of inducible NO synthase (iNOS) gene expression on diastolic function changes. **Methods.** In 36 HTx, FPs were measured before and after volume load, following which Starling curves were constructed using PCWP and cardiac index (CI). Patients were categorized into those with normal (group A, n = 21) and abnormal hemodynamics (group B, n = 15, PCWP >15 mm Hg at rest or >18 mm Hg following volume load). For the establishment of the potential role of NO, endomyocardial iNOS gene expression level was measured. **Results.** Except for PCWP ($P < 0.001$) and mean pulmonary artery pressure ($P < 0.001$) no differences in age, baseline characteristics, and ejection fraction were observed between both groups, and volume load significantly increased PCWP in both groups (group A: $P < 0.001$ and group B: $P < 0.001$) without any change in heart rate. Interestingly, volume load significantly increased CI in group A ($P < 0.001$) but not in group B ($P = 0.654$), and the Starling curves revealed a higher CI at any given PCWP in group A together with significantly higher iNOS gene expression ($P = 0.009$). **Conclusions.** In HTx, volume load increases FP and unmasks the presence of left ventricular diastolic dysfunction. Interestingly, following saline load group B shows a blunted Starling response, with higher PCWP and lack of CI increase at any given PCWP. The higher iNOS gene expression level in group A suggests a potential role of NO as mediator of diastolic function.

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INTRODUCTION

Cardiac transplantation is an established therapy for patients with end-stage heart failure refractory to medical therapy. Although transplant recipients have a good exercise capacity, this capacity is often subnormal compared with that of healthy controls,[1,2] even in the presence of a normal left ventricular (LV) ejection fraction (EF), independent of the presence of cardiac allograft vasculopathy. Recent studies suggest that, in addition to cardiac denervation, chronotropic incompetence, and subclinical rejection episodes, abnormalities in the diastolic function of the transplanted heart with an increase in the degree of myocardial stiffness and reductions in the level of cycle efficiency with incoordinate contraction and relaxation play a major role in impaired exercise tolerance. These changes in diastolic distensibility are associated with altered inducible nitric oxide synthase (iNOS) gene expression. In this regard, it has been demonstrated that the intracoronary infusion of nitric oxide (NO) donors increases the rate of LV relaxation and degree of LV diastolic distensibility without affecting patients' LV EF or dP/dtmax.[3-6]

Although the rate of LV relaxation and passive chamber stiffness can be determined directly using a multiple-loop conductance

catheter to record the end-diastolic pressure-volume relationship during single beat or ideally by means of multibeat pressure-volume loops during preload modulation,[7] right heart catheterization is usually considered for the structured workup of diastolic dysfunction.[8] Diastolic dysfunction can be diagnosed at a resting pulmonary capillary wedge pressure (PCWP) ≥15 mm Hg, as measured using the Swan-Ganz catheter, in the presence of a normal LV end-diastolic volume index[9]; however, although patients with early-stage disease often display a normal PCWP at rest, they show abnormal hemodynamics when the cardiovascular system is subjected to stress by exercise or rapid saline infusion. Therefore, as a normal resting PCWP does not necessarily exclude diastolic dysfunction, exercise right heart catheterization and rapid volume load infusion have been recommended as testing modalities for the work up of diastolic dysfunction.[8] A steep increase in the PCWP during exercise or a peak exercise PCWP ≥25 mm Hg, or a PCWP >18 mm Hg following rapid saline infusion is considered abnormal.[8,10-13] Therefore, according to recent recommendations, one should consider provocative testing in patients presenting with a baseline PCWP of 13 to 15 mm Hg and pulmonary hypertension to differentiate those with isolated pulmonary arterial hypertension from those with pulmonary arterial hypertension due to heart failure with preserved EF (HFpEF).

There is currently a lack of data on the hemodynamic effects of rapid volume loading in heart transplant (HTx) recipients. Accordingly, this study aimed to investigate the hemodynamic changes that occur following acute volume load in HTx patients and evaluate whether acute volume load has the potential to unmask abnormal hemodynamics suggestive of "early" diastolic dysfunction in this population. Additionally, we sought to investigate the potential contribution of NO to the observed differences in the LV contractile performance quality by measuring endomyocardial iNOS gene expression.

MATERIALS AND METHODS

Study Population

We prospectively enrolled asymptomatic HTx recipients scheduled for elective left and right heart catheterization at the time of an annual diagnostic checkup. The mean patient age was 63 ± 13 y. All patients were receiving immunosuppressive therapy, comprising various combinations of cyclosporine, prednisone, mycophenolate, sirolimus, and azathioprine. For ethical reasons, the administration of immunosuppressive and antihypertensive therapies was maintained during the study. Coronary angiography, which preceded the study protocol, revealed the presence of angiographically normal coronary arteries without evidence of accelerated graft atherosclerosis. None of the patients had biopsy-obtained evidence of rejection (International Society of Heart and Lung Transplantation acute cellular classification greater than or equal to grade 1R1 and pathological antibody-mediated rejection classification greater than or equal to grade 1) requiring immunosuppressive therapy adjustment at the time of the study. Informed consent was obtained from all patients, and the study protocol was approved by the local ethics committee. No complications related to the procedure were observed.

Patients were categorized according to their baseline hemodynamic values and response to rapid saline infusion. Those with normal hemodynamics, as characterized by a PCWP ≤15 mm Hg at rest and ≤18 mm Hg following volume load, were assigned to group A, whereas those with abnormal hemodynamics, as characterized by a PCWP >15 mm Hg at rest or >18 mm Hg following volume load, were assigned to group B.[11-15]

Cardiac Catheterization Data and Saline Infusion Protocol

After the exclusion of significant graft vasculopathy, a 7-F Swan-Ganz catheter was positioned under fluoroscopic guidance for the measurement of right atrial pressure (RAP), PCWP, systolic pulmonary artery pressure (PA), diastolic PA, and mean PA (PAmean) at end expiration. The correct position of the Swan-Ganz catheter was confirmed by fluoroscopy and the presence of characteristic pressure waveforms.[11,14] A PCWP saturation >90% was obtained to confirm the correct position of the Swan-Ganz catheter in wedge position. All pressure tracings were digitized and stored for offline analysis. Cardiac output (CO) was measured using thermodilution as the average of 3 measurements with <10% variance and indexed to the body surface area (cardiac index [CI]). Stroke volume (SV) was determined by the CO divided by the heart rate and indexed for the body surface area (SV index [SVi]). LV transmural pressure (LVTMP), which reflects the LV preload, independent of the right heart filling pressure (FP) and pericardial constraint, was estimated as the PCWP-RA.[15,16] The obtained PCWP and CI data were used for estimation of the Starling (CI/PCWP) relationship. The LV stroke work index (LVSWi) was calculated as follows: (mean arterial blood pressure – PCWP) × SVi × 0.0136. The total peripheral resistance index was calculated as (brachial mean blood pressure – RAP) × 80/CI. Transpulmonary gradient was calculated as the PAmean minus PCWP, whereas the pulmonary vascular resistance index was determined as the transpulmonary gradient multiplied by 80 and divided by the CI.

All hemodynamic measurements were obtained at baseline and immediately after the intravenous administration of 7 mL/kg of saline through the sidearm of an 8 French sheath canula placed in the femoral vein over 5 to 10 min, facilitated by the use of a pneumatic sleeve compressing the infusate, as previously described.[17]

Biomarkers

Blood samples for laboratory assessments were obtained from the patients at the start of the study. Blood (10 mL) was drawn from the femoral vein. Plasma was separated via centrifugation at 1500*g* for 10 min at 4 °C and transferred into 1-mL cryotubes for storage at −70 °C for later analysis. Circulating galectin-3 and ST2 levels were determined using a commercially available ELISA kit (Biomérieux Clinical Diagnostic, Firenze, Italia, and Presage ST2 Assay, Critical Diagnostics, CA) according to the manufacturer's instructions. The level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) was measured using electrochemiluminescence technology (Cobas 6000e501, Roche Diagnostics, Mannheim, Germany). We also determined the patients' blood hemoglobin, high-sensitivity cardiac troponin, and creatinine concentrations using routine techniques (Cobas Integra 800 Roche Diagnostics, Mannheim, Germany). Estimated glomerular filtration rate was used as an indicator of renal function and was estimated from serum creatinine.[18]

Quantitative Real-time Reverse Transcriptase Polymerase Chain Reaction

At the end of the study, all patients underwent right ventricular (RV) endomyocardial biopsy. The biopsy samples

were immediately frozen in liquid nitrogen and stored at −80 °C for the subsequent determination of iNOS gene expression using highly sensitive reverse transcriptase polymerase chain reaction (RT-PCR), as previously described.[19] Briefly, total RNA was isolated from RV endomyocardial biopsies using the RNeasy Fibrous Tissue Mini Kit (Qiagen) and digested with DNase. RNA was reverse transcribed with random primers using the High-Capacity cDNA Archive Kit (Applied Biosystems). RT-PCR was performed in 96-well plates on an ABI Prism 7000 Sequence Detection System using TaqMan Universal PCR Master Mix and Assay-On-Demand (iNOS assay ID Hs01075529_m1), with a final reaction volume of 25 µL. All samples were analyzed in triplicate. The relative expression of the target gene was normalized to the level of GAPDH.[19]

Statistical Analysis

Continuous variables with a normal distribution are presented as mean ± SD and nonnormally distributed variables as median (interquartile range). Categorical variables are presented as percentages. *T* tests or Mann-Whitney *U* tests were used according to the distribution of the variables for between-group comparisons. A *P* value <0.05 was considered statistically significant. Univariate and multivariate linear regression were used to predict iNOS2 gene expression levels. Univariate parameter estimates with $P < 0.1$ significance level were considered further in a multivariate model. In case of high correlation between 2 possible predictors, only one of the correlated pairs was used for model selection. The following parameters were included in the univariate model: age, gender, years after HTx, heart rate, PAmean, transpulmonary gradient, SVi, LVTMP,

TABLE 1.

Baseline characteristics of the study population

	All (n = 36 patients)	Group A (n = 21 patients)	Group B (n = 15 patients)	*P*
Demography				
BSA, m^2	1.90 ± 0.21	1.90 ± 0.16	1.91 ± 0.29	0.147
Age recipient, y	63 ± 13	62 ± 14	63 ± 12	0.868
Age donor, y	32 ± 14	34 ± 13	30 ± 12	0.473
Years post-HTx, y	9.3 ± 8.9	7.4 ± 6.8	11.1 ± 10.5	0.199
Male, %	78	76	80	0.456
Hypertension, %	59	57	60	0.673
Immunosuppressive regimen				
Tacrolimus, %	49	51	48	0.357
Cyclosporine, %	51	49	52	0.482
mTOR inhibitors, %	15	14	16	0.473
Mycophenolate mofetil, %	67	72	61	0.162
Azathioprine, %	7	6	9	0.156
Rejection				
Acute cellular rejection ≥2R, n (%)	6 (17)	4 (19)	2 (13)	0.256
Antibody-mediated rejection, n (%)	3 (8)	2 (9)	1 (7)	0.412
LV hemodynamics				
LVEDVI, mL/m^2	65 ± 19	71 ± 22	58 ± 14	0.258
EF, %	60 ± 12	63 ± 10	57 ± 13	0.310
HR, beats/minute	83 ± 10	82 ± 9	84 ± 12	0.487
Aomean, mm Hg	101 ± 17	96 ± 16	107 ± 15	0.061
RAP, mm Hg	4.9 ± 3.9	3.8 ± 2.8	6.5 ± 4.8	0.035
PCWP, mm Hg	10.2 ± 4.7	7.9 ± 2.9	13.6 ± 4.9	<0.001
PAmean, mm Hg	16.9 ± 5.8	14.2 ± 3.9	20.9 ± 6.0	<0.001
TPG, mm Hg	6.7 ± 2.0	6.3 ± 1.9	7.3 ± 2.2	0.162
PVRI, $dynes/s/cm^5/m^2$	187 ± 64	182 ± 68	195 ± 68	0.546
LVTMP, mm Hg	5.3 ± 3.0	4.1 ± 1.8	7.1 ± 3.6	0.002
RAP/PCWP	0.45 ± 0.23	0.45 ± 0.22	0.44 ± 0.25	0.942
CI, $L/min/m^2$	3.0 ± 0.7	2.9 ± 0.8	3.0 ± 0.5	0.657
SVi, $mL/min/m^2$	36.7 ± 9.0	36.8 ± 10.9	36.7 ± 6.3	0.986
LVSWi, $gm\text{-}m/m^2/beat$	44.72 ± 12.17	43.03 ± 12.64	46.59 ± 11.97	0.406
Labo parameters				
NT-proBNP, pg/mL	1983 ± 4323	952 ± 2486	3634 ± 6085	0.040
Creat, mg/L	1.51 ± 0.62	1.35 ± 0.49	1.65 ± 0.68	0.118
eGFR, mL/min	51 ± 21	56 ± 20	45 ± 19	0.136
ST2, ng/mL	32.5 ± 23.1	22.8 ± 8.9	46.4 ± 30.8	0.002
hsTnT, ng/L	30.8 ± 30.7	16.6 ± 14.7	49.4 ± 37.3	0.001
Galectin-3, ng/mL	12.8 ± 7.6	10.6 ± 4.9	15.3 ± 9.9	0.085
Gene expression				
iNOS gene expression (arbitrary units)	0.000308 ± 0.000196	0.00387 ± 0.000196	0.000215 ± 0.000145	0.009

Aomean, mean aortic pressure; BSA, body surface area; CI, cardiac index; Creat, creatinine; EF, ejection fraction; eGFR, estimated glomerular filtration rate; HR, heart rate; HTx, heart tansplantation; iNOS, inducible nitric oxide synthase; LV, left ventricular; LVEDVI, left ventricular end-diastolic volume index; LVSWi, left ventricular stroke work index; LVTMP, left ventricular transmural pressure; mTOR, mechanistic target of rapamycin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAmean, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; PVRI, pulmonary vascular resistance index; RAP, right atrial pressure; SVi, stroke volume index; SVRI, systemic vascular resistance index; TPG, transpulmonary gradient.

abnormal hemodynamics (group B), change in PAmean and SVi following volume load. Graphical assessment was used to check the normality assumption of the residuals.

All analyses were performed using IBM SPSS Statistics, version 25, software (IBM Corp) and Prism 7.0 software (GraphPad Software Inc) or calculated in R environment (R Foundation for Statistical Computing) software, version 3.4.1.0.

RESULTS

Baseline Characteristics and Resting Hemodynamics

Right heart catheterization with fluid challenge was performed in 40 patients during the study period. Four patients were excluded from this study because accurate end-expiratory PCWP tracings could not be performed; total, 36 patients were included in the final analysis. Twenty-one (59%) and 15 (41%) patients, respectively, were classified as having either normal or elevated FPs. The baseline clinical and hemodynamic characteristics of the study population are summarized in Table 1. No differences in the immunosuppressive regimen and number of preceding rejection episodes were observed between the groups. All patients were strictly asymptomatic.

Six patients had elevated FPs at the baseline, and 9 who were initially classified as having a normal FP, with a PCWP <15 mm Hg, fulfilled the criteria after fluid challenge, resulting in a total of 15 patients in the group with abnormal hemodynamics (group B; Table 2). Baseline characteristics of group B

TABLE 2.

Baseline characteristics of group B patients with and without abnormal filling pressures at baseline

	Group B (n = 15 patients)	PCWP ≤15 mm Hg (n = 9 patients)	PCWP >15 mm Hg (n = 6 patients)	*P*
Demography				
BSA, m^2	1.91 ± 0.29	1.92 ± 0.32	1.98 ± 0.25	0.816
Age recipient, y	63 ± 12	63 ± 11	63 ± 14	0.991
Age donor, y	30 ± 12	39 ± 10	32 ± 14	0.387
Years post-HTx, y	11.1 ± 10.5	8.3 ± 7.8	15.2 ± 13.4	0.226
Male, %	80	78	83	0.456
Hypertension, %	60	66	50	0.097
Immunosuppressive regimen				
Tacrolimus, %	48	44	50	0.245
Cyclosporine, %	52	55	50	0.473
mTOR inhibitors, %	16	11	17	0.359
Mycophenolate mofetil, %	61	55	67	0.162
Azathioprine, %	9	0	17	0.482
Rejection				
Acute cellular rejection ≥2R, n (%)	2 (13)	1	1	0.256
Antibody-mediated rejection n (%)	1 (7)	0	1	0.412
LV hemodynamics				
LVEDVI, mL/m^2	58 ± 14	56 ± 17	62 ± 6	0.716
EF, %	57 ± 13	57 ± 6	54 ± 18	0.142
HR, beats/min	84 ± 12	83 ± 8	86 ± 17	0.577
Aomean, mm Hg	107 ± 15	109 ± 13	103 ± 15	0.38
RAP, mm Hg	6.5 ± 4.8	4.6 ± 3.0	9.5 ± 5.8	0.049
PCWP, mm Hg	13.6 ± 4.9	10.6 ± 3.0	18.2 ± 3.5	0.001
PAmean, mm Hg	20.9 ± 6.0	17.4 ± 3.8	26.1 ± 4.9	0.002
TPG, mm Hg	7.3 ± 2.2	6.9 ± 1.6	7.9 ± 2.9	0.385
PVRI, $dynes/s/cm^5/m^2$	195 ± 68	97 ± 23	115 ± 43	0.331
LVTMP, mm Hg	7.1 ± 3.6	6.0 ± 19	8.7 ± 4.9	0.164
RAP/PCWP	0.44 ± 0.25	0.40 ± 0.22	0.51 ± 0.30	0.419
CI, $L/min/m^2$	3.0 ± 0.5	3.1 ± 0.6	3.0 ± 0.4	0.828
SVi, $mL/min/m^2$	36.7 ± 6.3	37.4 ± 6.4	35.8 ± 6.5	0.643
LVSWi, $gm\text{-}m/m^2/beat$	46.59 ± 11.97	50.10 ± 11.30	41.36 ± 11.87	0.177
Labo parameters				
NT-proBNP, pg/mL	3634 ± 6085	880 ± 621	7307 ± 8211	0.045
Creat, mg/L	1.65 ± 0.68	1.50 ± 0.43	1.88 ± 0.94	0.304
eGFR, mL/min	45 ± 19	47 ± 18	43 ± 22	0.667
ST2, ng/mL	46.4 ± 30.8	53.9 ± 39.3	36.3 ± 10.0	0.309
hsTnT, ng/L	49.4 ± 37.3	42.6 ± 37.7	60.3 ± 38.0	0.428
Galectin-3, ng/mL	15.3 ± 9.9	15.4 ± 9.9	15.2 ± 10.8	0.970
Gene expression				
INOS gene expression (arbitrary units)	0.000215 ± 0.000145	0.000276 ± 0.000160	0.000130 ± 0.000300	0.087

Aomean, mean aortic pressure; BSA, body surface area; CI, cardiac index; Creat, creatinine; EF, ejection fraction; eGFR, estimated glomerular filtration rate; HR, heart rate; HTx, heart tansplantation; iNOS, inducible nitric oxide synthase; LV, left ventricular; LVEDVI, left ventricular end-diastolic volume index; LVSWi, left ventricular stroke work index; LVTMP, left ventricular transmural pressure; mTOR, mechanistic target of rapamycin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAmean, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; PVRI, pulmonary vascular resistance index; RAP, right atrial pressure; SVi, stroke volume index; SVRI, systemic vascular resistance index; TPG, transpulmonary gradient.

patients with and without abnormal FPs at baseline are summarized in Table 2.

As expected, per definition, right and left heart filling and PA pressures were significantly higher in the group with elevated LV FP (group B) than in the normal LV FP group (group A; RAP: $P = 0.035$, PCWP: $P < 0.001$, and PAmean: $P < 0.001$), whereas the transpulmonary gradient, CI, and SVi were similar in both groups.

Interestingly, the blood samples revealed significantly higher NT-proBNP, ST2, and high-sensitivity cardiac troponin levels in the patients with an elevated LV FP than in those with a normal value.

Hemodynamic Response to Rapid Saline Infusion

Table 3 summarizes the baseline and postfluid hemodynamic values in the patients with and without abnormal hemodynamic parameters.

Rapid saline infusion induced a significant increase in the PCWP, meanPA, RAP, and LVTMP in each group without affecting the heart rate, mean aortic pressure, and RAP/PCWP. Although the CI during saline infusion remained unchanged in group B secondary to limitations in the rise in SVi and invariable heart rate, a significant increase in the value secondary to a rise in the SVi, not heart rate, was observed in group A. No differences in LVTMP were noted between both groups following volume load.

Volume Load and Starling Relationship

In group B, the Starling relationship showed a lower CI at any given PAmean, PCWP, and LVTMP during saline infusion and significant disparities in the degree of increase in the PCWP, LVTMP relative to the CI (P <0.01) with the group B patients showing a steeper PCWP/CI, PAmean/CI, and LVTMP/CI slope than the group A patients (Figure 1). In addition, the LVSWi remained unchanged in group B, whereas a significant rise in the value, from 43.03 ± 12.64 to 48.40 ± 12.12 g/mL/beat/m^2, was noted in group A ($P = 0.002$; Table 2). In group A, this increase in the LVTMP was coupled with an increase in LV stroke work, compatible with a rise in LV performance, whereas opposite changes were observed in group B, in which, despite a rise in the FPs and LVTMP, LVSWi did not increase and remained unchanged following volume load (Figure 2).

Volume Load and iNOS Gene Expression

The patients in group B were characterized by a significantly lower endomyocardial iNOS gene expression level (Figure 3). We performed a linear regression analysis to examine whether iNOS gene expression is associated with baseline characteristics, hemodynamics, and the observed hemodynamic changes following volume load. Using the univariate model recipient age, gender, PAmean, and the presence of abnormal hemodynamics were significant predictors of iNOS2 gene expression. In the multivariate model, only recipient age and abnormal hemodynamics remained independent predictors (Table 4).

DISCUSSION

In this study, we reported, for the first time, that a subgroup of asymptomatic HTx patients displays a greater dependence on plasma volume expansion by saline infusion. Apart from showing a more extensive increase in the FP, these HTx patients are characterized by a blunted Frank-Starling relationship, with no increase in the SV for any given rise in the FP. We speculate that the lower iNOS gene expression level compared with that in those with a normal hemodynamic response may have resulted in an impaired NO-mediated right and downward shift of the diastolic pressure-volume relationship and a lack of increase in the LV preload recruitment following volume load. In addition, the reduced LVSWi following volume load suggests that, in these HTx patients, an impaired ventricular reserve may also partially contribute to an abnormal hemodynamic response.

Unmasking Abnormal Hemodynamic Response With Volume Load

Patients with diastolic dysfunction may tend to be missed if the diagnosis relies exclusively on resting echocardiographic and hemodynamic parameters. Therefore, owing to the poor sensitivity of resting parameters, contemporary algorithms[20-22] have introduced the implementation of a volume challenge or right heart catheterization with exercise testing[11,23] in the

TABLE 3.

Hemodynamics at baseline and following volume load in patients with (group B) and without (group A) abnormal hemodynamics

	Group A (normal response)			Group B (abnormal response)		
	Baseline	Volume load	*P*	Baseline	Volume load	*P*
Aomean, mm Hg	96 ± 16	98 ± 14	0.134	107 ± 15	108 ± 15	0.658
HR, beats/min	82 ± 9	80 ± 9	0.178	84 ± 12	84 ± 12	0.938
RAP, mm Hg	3.8 ± 2.8	6.7 ± 3.0	<0.001	6.5 ± 4.8	10.5 ± 4.9	<0.001
PAmean, mm Hg	14.2 ± 3.9	21.2 ± 4.4	<0.001	20.9 ± 6.0	28.3 ± 6.4	0.003
PCWP, mm Hg	7.9 ± 2.9	13.2 ± 3.5	<0.001	13.6 ± 4.9	21.3 ± 4.2	<0.001
CI, L/m^2	2.9 ± 0.8	3.2 ± 0.6	<0.001	3.0 ± 0.5	3.1 ± 0.6	0.654
SVi, mL/m^2	36.8 ± 10.9	41.4 ± 9.0	<0.001	36.7 ± 6.3	37.5 ± 7.8	0.505
TPG, mm Hg)	6.3 ± 1.9	8.1 ± 2.4	0.027	7.3 ± 2.2	7.0 ± 3.9	0.793
LVTMP, mm Hg	4.1 ± 1.8	6.7 ± 3.6	0.002	7.1 ± 3.6	10.7 ± 5.1	0.006
RAP/PCWP	0.45 ± 0.22	0.52 ± 0.22	0.198	0.44 ± 0.25	0.49 ± 0.22	0.264
LVSWi, g/mL/beat/m^2	43.03 ± 12.64	48.40 ± 12.12	0.002	46.59 ± 11.97	44.89 ± 13.11	0.250

Aomean, mean aortic blood pressure; CI, cardiac index; HR, heart rate; LVSWi, left ventricular stroke work index; LVTMP, left ventricular transmural pressure; PAmean, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; SVi, stroke volume index; TPG, transpulmonary gradient.

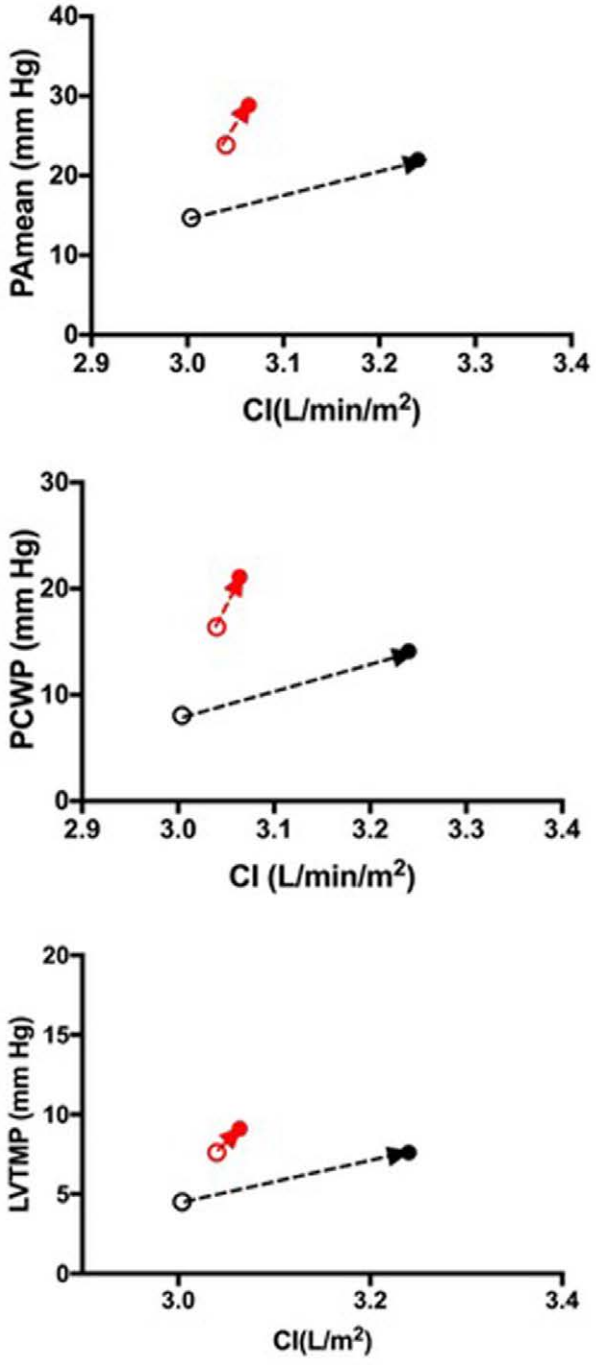

FIGURE 1. Frank-Starling relationship in patients with normal (group A; black) and abnormal hemodynamics (group B; red). Open dots represent baseline cardiac index (CI)/pulmonary capillary wedge pressure (PCWP) relationship; closed dots represent relationship following volume load. LVTMP, left ventricular transmural pressure; PAmean, mean pulmonary artery pressure.

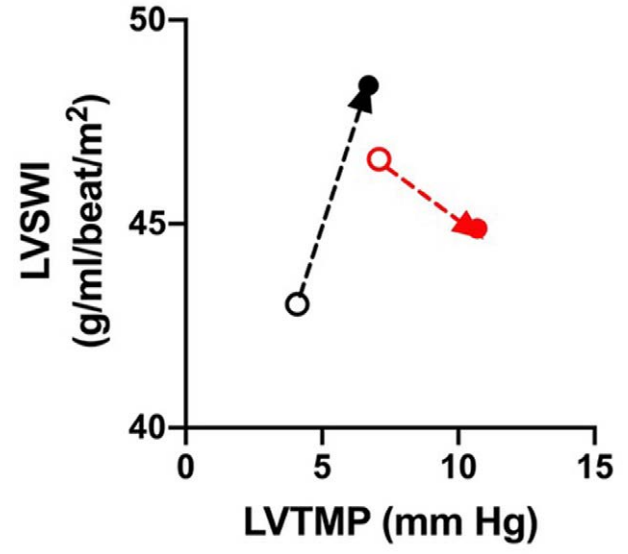

FIGURE 2. Left ventricular stroke work index (LVSWi)-left ventricular transmural pressure (LVTMP) relationship in patients with normal (group A; black) and abnormal hemodynamics (group B; red). Open dots represent baseline LVSWi-LVTMP relationship; closed dots represent relationship following volume load.

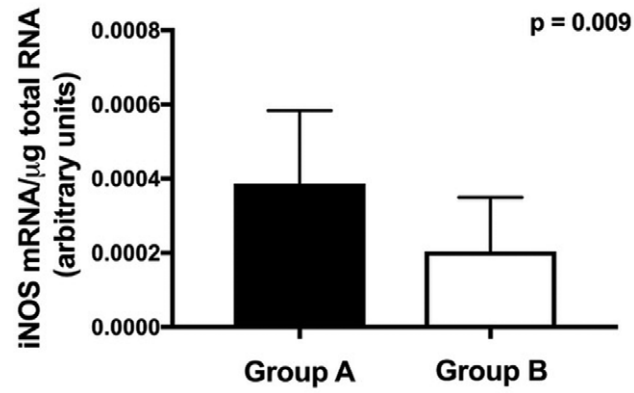

FIGURE 3. Inducible nitric oxide synthase (iNOS) gene expression in patients with (group B) and without (group A) abnormal hemodynamics.

hemodynamic assessment of diastolic function.[9,21-23] The advantage of volume challenge over invasive exercise testing is that it is widely available and can easily be performed in the laboratory. It has minimal effects on patients' blood pressure and heart rate and predominantly tests the degree of operant ventricular compliance.[24,25] Similar to the findings of Meluzin et al[26] and Clemmensen et al,[27] who performed invasive exercise testing in a HTx population with a preserved LV EF, 17% of our group B patients had a resting PCWP >15 mm Hg, and 9 patients could be reclassified, following the volume challenge, as having an abnormal hemodynamic response suggestive of an impaired diastolic reserve. This similarity with previous observations emphasizes that, in an HTx population, the assessment of elevated LV FPs during resting conditions as a measure for the evaluation of myocardial diastolic performance falls short and that, similar to HFpEF cases,[28] volume loading needs to be performed for the proper assessment of hemodynamics.

Data about the prognostic role of biomarkers in HTx patients are scarce. Unfortunately, the design of the present study did not allow for the evaluation of the prognostic implication of elevated LV FPs; however, the levels of surrogate biomarkers of inferior prognoses, such as ST2,[29] NT-proBNP,[30] and high-sensitive troponin,[31] were higher in patients with an abnormal hemodynamic response.

Volume Load and LV Performance

During volume load, all the HTx patients displayed an increase not only in the LV and RV FP but also in the LVTMP,

TABLE 4.

Univariate and multivariate predictors of iNOS gene expression

	Univariate models		Final multivariate model	
	Coefficient (95% CI)	*P*	Coefficient (95% CI)	*P*
Demographics				
Age, y	−0.07 (−0.12 to −0.02)	<0.01	−0.06 (−0.10 to −0.02)	<0.01
Gender (male)	1.70 (−0.0 to 3.44)	0.057	NA	NS
Years post-HTx	−0.13 (−0.21 to −0.05)	<0.01	NA	NS
PAmean, mm Hg	−0.13 (−0.46 to −0.03)	<0.05	NA	NS
Group B	−1.72 (−3.09 to −0.34)	<0.05	−1.60 (−2.7 to −0.43)	<0.05

CI, confidence interval; HTx, heart transplant; iNOS, inducible nitric oxide synthase; NA, not available; NS, nonsignificant; PAmean, mean pulmonary artery pressure.

reflecting the ability of the pericardium to actively accommodate the volume infused. Moreover, this increase in the LVTMP was not accompanied by a significant change in the RAP/PCWP ratio, indicating that both LV and RV congestion contributed equally to the increase in the FP.

In group A, this increase in the LVTMP was coupled with an expected increase in the SV and LV stroke work, compatible with a rise in LV performance, as evidenced by the positive relationship between the stroke work index and LVTMP. Opposite changes were observed in group B, in which a rise in the FPs and LVTMP was observed, similar to those in group A; however, the SVi did not increase and remained unchanged following volume load. Furthermore, the absence of any change in the SV was coupled with an impaired LV reserve and a blunted LV stroke work augmentation ability. Pericardial constraint and ventricular interaction as potential mechanisms for this observation can be excluded because these apparent changes persist when PCWP is substituted for LVTMP. Therefore, in contrast to patients with diastolic dysfunction due to obesity and exercise-induced pulmonary hypertension where pericardial constraint is increased and ventricular interdependence enhanced,[15] the lower SV and CO following volume load might at least partially reflect an abnormal end-diastolic PV relationship in this subgroup of HTx patients.

Volume Load and Diastolic Function: Role of iNOS

In the normal heart, the increased cardiac work observed following volume load is achieved by the enhancement of relaxation and suction. In this regard, blood is effectively pulled from the left atrium into the compliant LV chamber, with little or no increase in the degree of passive chamber stiffness. In diastolic dysfunction, this mechanism fails, and the LV diastolic pressure-volume relationship is shifted upward and to the left, indicating an increase in the passive chamber stiffness level, which, in turn, is responsible for the rise in the LV FPs.

NO and its bioavailability play a major role in cardiac lusitropy. Among athletes and in patients with heart failure with reduced and preserved EF, NO enhances the rate of LV preload recruitment by pushing down and rightward the filling curves.[32] Of note, in patients with nonischemic dilated cardiomyopathy[33] and in HTx recipients,[34,35] an increase in the level of iNOS gene expression or the intracoronary infusion of substance P, which releases NO from the coronary endothelium, augments the LV SV and LV stroke work values without affecting the quality of systolic function. Similarly, in human and in animal studies, enhanced cyclic guanosine monophosphate availability through phosphodiesterase 5 inhibitor-mediated NO production beneficially affects diastolic properties of the failing heart.[36] Furthermore, it has recently been demonstrated that the increase in the cardiac FP, pulmonary hypertension, and inadequate CO reserve observed in HFpEF patients following exercise can be attenuated by the inhalation of NO or by an acute infusion of sodium nitrite.[37,38] The higher FPs and the absence of any increase in the SV and stroke work index observed in those with lower iNOS gene expression corroborate these observations. As shown in the Frank-Starling curve, the HTx patients with elevated LV FP values had a steeper PCWP/CI relationship, compatible with a left and upward shift of the PV relationship. Therefore, we speculate that the weaker iNOS gene expression in this group of patients might at least partially account for a blunted NO-mediated rightward shift of the diastolic pressure-volume relationship and might, therefore, be responsible for the impaired LV preload reserve/recruitment. Although dysregulated expression and activity of NOS isoforms appear to contribute to diastolic dysfunction, the exact mechanisms that regulate the expression and activity of NOS isoforms are not well understood and have been related to endothelial dysfunction and inflammation.

Limitations

This study has some limitations that must be acknowledged. First, it had a single-center design and a small sample size, owing to which the presence of type I errors cannot be ruled out. The results and conclusions should be considered as hypothesis generating only. Second, it should be noted that, at present, there is a lack of consensus on how a fluid challenge is best performed and interpreted, as well-standardized PCWP cutoff for pathologic response to salt loading is lacking. To account for this uncertainty, the study data were analyzed using a threshold of 18 mm Hg, which was proposed as an index for the detection of pulmonary hypertension due to left heart disease.[13] Nevertheless, there is a need for the additional standardization of fluid-loading protocols.[39] Third, ours was an invasive study. We opted for an invasive approach because it has been shown especially in HTx patients that the noninvasive assessment of LV FP by echocardiographic Doppler measurement is poorly correlated with invasive pressure measurement.[26,40] Furthermore, the presence of iNOS in the myocardium was established by the demonstration of iNOS mRNA and not by the direct myocardial demonstration of the iNOS protein itself, which would provide definite proof of myocardial iNOS presence as a result of the post-transcriptional modification of iNOS protein translation[41]; however, a previous study demonstrated the presence of iNOS protein immunostaining in 80% of all myocardial biopsies

that showed iNOS mRNA expression by RT-PCR.[19] Finally, coronary allograft vasculopathy was evaluated solely by angiography, which is less sensitive to detect early stages of cardiac allograft vasculopathy than intravascular ultrasound or optical coherence tomography.

Clinical Perspectives

The direct invasive measurement of LV filling at rest and during volume load aided in the identification of an elevated LV FP in approximately 42% of the asymptomatic HTx patients. Such HTx patients not only display a different biomarker profile but also unique pathophysiologic features, including more profound hemodynamic derangements and a blunted Frank-Starling relationship. Interestingly, these abnormalities were related to lower iNOS gene expression levels; however, as all patients were asymptomatic at time of examination, the clinical impact of these findings on outcome remains undetermined. Therefore, additional studies are required not only to delineate the underlying cellular and mechanical abnormalities responsible but also to define the clinical implications of these observations and explore the role of novel therapies.

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