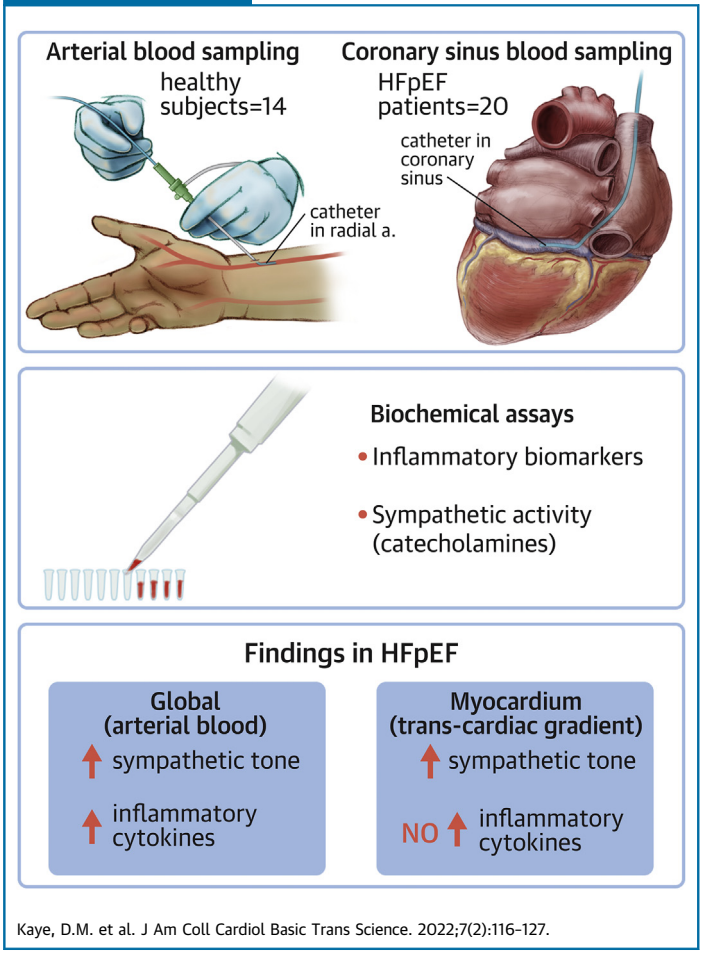
JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 7, NO. 2, 2022

CLINICAL RESEARCH

Characterization of Cardiac Sympathetic Nervous System and Inflammatory Activation in HFpEF Patients

David M. Kaye, MD, PhD,[a,b,c] Shane Nanayakkara, MD, PhD,[a,b] Bing Wang, PhD,[b,c] Waled Shihata, PhD,[a] Francine Z. Marques, PhD,[d] Murray Esler, MD, PhD,[e] Gavin Lambert, PhD,[f] Justin Mariani, MD, PhD[a,b,c]

VISUAL ABSTRACT



Kaye, D.M. et al. J Am Coll Cardiol Basic Trans Science. 2022;7(2):116-127.

HIGHLIGHTS

- **Although there is evidence for activation of the sympathetic nervous system and inflammatory pathways in peripheral blood samples, their relationship to myocardial activity is unknown.**
- **Using arterial and coronary sinus blood sampling, we have shown the presence of cardiac and systemic sympathetic activation in HFpEF patients. However although systemic inflammatory activation was readily apparent, there was detectable myocardial release of inflammatory cytokines.**
- **Key hemodynamic and demographic factors that typically cluster together in HFpEF appeared to drive cardiac sympathetic activation.**
- **The data suggest that there may be a role for antiadrenergic therapies in selected HFpEF patients.**

From the [a]Heart Failure Research Group, Baker Heart and Diabetes Institute, Melbourne, Australia; [b]Department of Cardiology, Alfred Hospital, Melbourne, Australia; [c]Department of Medicine, Monash University, Melbourne, Australia; [d]Hypertension Research Laboratory, School of Biological Sciences, Monash University, Melbourne, Australia; [e]Human Neurotransmitter Laboratory, Baker Heart and Diabetes Institute, Melbourne, Australia; and the [f]Iverson Health Innovation Research Institute and School of Health Science, Swinburne University of Technology, Melbourne, Australia.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2021.11.007

SUMMARY

We have shown that systemic and cardiac sympathetic activation is present in heart failure with preserved ejection fraction (HFpEF) patients. Conversely, whereas systemic inflammatory activation was also detected in HFpEF, we did not detect local myocardial release of inflammatory cytokines. Activation of the sympathetic system correlated with both hemodynamic and demographic factors that characteristically cluster together in HFpEF. Together these data suggest that there may be a role for antiadrenergic therapies in certain HFpEF patients. The study does not implicate locally derived cytokines in the myocardial biology of HFpEF, although systemic sources may contribute to the global pathophysiology of HFpEF. (J Am Coll Cardiol Basic Trans Science 2022;7:116–127)

ABBREVIATIONS AND ACRONYMS

BMI = body mass index

HFpEF = heart failure with preserved ejection fraction

NE = norepinephrine

PCWP = pulmonary capillary wedge pressure

SBP = systolic blood pressure

TC_{NE} gradient = transcardiac NE gradient

Heart failure with preserved ejection (HFpEF) continues to present one of the key contemporary diagnostic and therapeutic challenges in cardiovascular medicine. The growth in HFpEF prevalence is driven by several well known risk factors including hypertension, aging, and obesity acting in conjunction with a range of comorbidities including chronic kidney disease and chronic pulmonary disease. Various interventions proven to be effective in heart failure with reduced ejection fraction (HFrEF) have been conducted in HFpEF with limited or no impact on primary outcomes.[1,2] For example, despite evidence for marked efficacy in HFrEF, neprilysin inhibition has been shown to benefit only a subset HFpEF patients with left ventricular ejection fraction (LVEF) at the lower range of normal.[3,4] Recently, sodium-glucose transport protein 2 inhibitors were shown to exert favorable effects in HFpEF patients, principally due to an impact on heart failure (HF) hospitalization[5]; however, the mechanism responsible for this action remains uncertain.

The limited efficacy of many of the therapies tested in HFpEF could be explained by the possibility that the biological target of the relevant intervention may not be a key contributor to the HFpEF pathophysiology of HFpEF. In comparison with our understanding of the biology of HFrEF, current data regarding HFpEF is limited.[2] In particular, data regarding the myocardial biology of human HFpEF is limited. Although some data regarding patterns of changes in key biomarkers are available in HFpEF patients, it is well known that direct correlations between peripheral plasma concentrations and myocardial release or myocardial pathophysiology cannot necessarily be drawn.[6,7] A lack of therapeutic action for a particular drug class may also be explained, in part, by other factors including an inability to reverse an advanced HF phenotype or effectiveness in particular subphenotype.[8] It is also likely that contributory comorbidities may also require concomitant attention. For example, it has been proposed that inflammation is a key contributor to the pathophysiology of HFpEF.[9]

Given the current uncertainty regarding aspects of the myocardial biology of HFpEF, we performed systemic and transcardiac blood sampling in HFpEF patients and healthy volunteers to investigate the relative activities of the sympathetic nervous system and inflammatory pathways. As such, our aim was to definitively characterize the role of these systems in HFpEF and to identify the key biological triggers for their activation.

METHODS

STUDY POPULATION. The study included 20 patients with HFpEF and 14 healthy volunteers. HFpEF patients were referred to the Department of Cardiology, Alfred Hospital, for evaluation of exertional dyspnea with a LVEF >50% and in which HFpEF was suspected clinically. Exclusion criteria included significant coronary artery disease which had not been revascularized; moderate of greater aortic or mitral valve disease; infiltrative, restrictive, or hypertrophic myocardial disease; pericardial constriction; or significant right ventricular disease. Patients with significant pulmonary disease including chronic

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received August 23, 2021; revised manuscript received November 12, 2021, accepted November 16, 2021.

obstructive pulmonary disease were also excluded. The diagnosis of HFpEF was confirmed by the presence of a pulmonary capillary wedge pressure (PCWP) ≥15 mm Hg at rest or ≥25 mm Hg during symptom-limited exercise, according to published guidelines.[10] Healthy volunteers were recruited from the general community and had no history of significant comorbidities including cardiovascular, pulmonary, other systemic diseases. The study was approved by the Alfred Hospital Research and Ethics Committee, and all participants provided written informed consent.

CARDIAC CATHETERIZATION. Studies were conducted in the nonfasted state and background medications were continued. A 7-F balloon-tipped pulmonary artery catheter (Edwards Lifesciences) was inserted through an introducer sheath placed in the right internal jugular or a brachial vein for measurement of right atrial pressure, pulmonary artery pressure (PAP), and PCWP. The wedge position was confirmed by fluoroscopy and pressure waveform, and the mean PCWP was measured at end-expiration. Cardiac output was measured using thermodilution with measurements taken in triplicate or from five readings for patients in atrial fibrillation. A 3-F radial or brachial artery cannula was inserted for blood pressure recording and blood sampling. Following baseline hemodynamic measurements, a catheter was positioned within the coronary sinus at least 2 cm proximal to the coronary sinus ostium, as confirmed fluoroscopically. Subsequently, simultaneous arterial and coronary sinus blood samples were drawn. Subjects then performed graded supine exercise with evaluation of PAPs and cardiac output as previously described.[11]

BIOCHEMICAL ASSAYS. Arterial and coronary sinus plasma catecholamine concentrations were determined by high-performance liquid chromatography with electrochemical detection as previously described, with an intra-assay coefficient of variation of 5%.[12] Plasma arterial and coronary sinus concentrations of 92 inflammatory proteins were determined using the Olink Inflammation biomarker panel (Olink Proteomics). This assay, reported in arbitrary units, uses proximity extension assay technology in which oligonucleotide-antibody probe pairs bind to specific protein targets, with subsequent detection by polymerase chain reaction, as previously described, with an intra-assay variability of up to 12%.[13,14]

STATISTICAL ANALYSIS. Continuous normally distributed data are presented as mean ± SEM, whereas non-normal data are presented as the median with 25th and 75th percentiles (Q1-Q3). Between-group comparisons were performed using the Student *t*-test or Mann-Whitney test. Pearson's correlation coefficients were determined to examine the association between normally distributed data. Where indicated, an analysis of covariance was conducted to examine the influence of key covariates including systolic blood pressure (SBP), PCWP, age, and body mass index (BMI) on between-group differences on relevant dependent variables. Given that the distribution of several cytokines was not normally distributed, the Mann-Whitney test was applied to the comparison of all cytokine data. To investigate for differences between healthy subjects and HFpEF patients in the expression of inflammatory markers in arterial blood and in their transmyocardial gradients, we used two complementary approaches. We compared inflammatory patterns between healthy subjects and HFpEF using principal component analysis (PCA). PCA provides an opportunity to reduce the complexity of the large number of inflammatory cytokines assayed and to explore whether within cohort patterns could be identified. Analysis of covariance was used to investigate for differences in the relationship between the first and second principal components. In addition, between-group comparisons of individual inflammatory markers were conducted using unpaired Student *t*-test with correction for multiple comparisons using the false discovery rate of Benjamini and Hochberg. A q value of <0.05 was considered to be statistically significant. Statistical analysis was performed using SPSS (version 26, SPSS Inc) and R version 3.6.1.

RESULTS

BASELINE CHARACTERISTICS AND HEMODYNAMIC PROFILES. As shown in Table 1, HFpEF patients were aged 70 ± 2 years compared to 53 ± 2 years in healthy subjects ($P < 0.001$) and the proportion of women (n = 13 of 20) was greater in HFpEF than in the healthy cohort (n = 4 of 14) ($P = 0.037$). HFpEF patients were heavier than the healthy subjects (34 ± 2 kg/m^2 vs 26 ± 1 kg/m^2, $P = 0.001$). Consistent with a diagnosis of HFpEF, atrial fibrillation, treated hypertension, and diabetes were prevalent comorbidities. HFpEF patients had evidence of significantly increased PAP and PCWP, with a nonsignificant trend towards lower cardiac index as shown in Table 1. HFpEF patients had poorer exercise tolerance (peak workload capacity: 50 ± 8 W vs 122 ± 13 W, $P < 0.001$). The peak exercise PCWP was markedly elevated in

TABLE 1 Clinical Profiles

	Healthy Subjects	HFpEF
Demographics		
Age, y	53 ± 2	70 ± 2[a]
Sex, M/F	10/4	7/13
BMI, kg/m^2	26 ± 1	34 ± 2[b]
Hypertension, %	–	65
Atrial fibrillation, %	–	55
Diabetes, %	–	25
Coronary disease, %	–	20
ACE/ARB, %	–	60
Beta blocker, %	–	35
Echocardiography		
LVEDVI, mL/m^2	57 ± 3	55 ± 3
LVEF, %	62 ± 1	63 ± 1
LV mass index	72 ± 4	95 ± 6[b]
LA volume index	30 ± 2	44 ± 3[b]
Hemodynamics		
HR, beats/min	62 ± 3	68 ± 3
MAP, mm Hg	92 ± 3	99 ± 5
SBP, mm Hg	134 ± 4	144 ± 5
DBP, mm Hg	74 ± 2	75 ± 4
RAP, mm Hg	6 ± 1	7 ± 1
mPAP, mm Hg	14 ± 1	22 ± 2[a]
sPAP, mm Hg	22 ± 1	35 ± 3[a]
dPAP, mm Hg	9 ± 1	14 ± 1[b]
PCWP, mm Hg	9 ± 1	13 ± 1[c]
Cardiac index, L/min/m^2	2.8 ± 0.2	2.5 ± 0.1
Ex PCWP, mm Hg	16 ± 1	31 ± 1[a]
Ex cardiac index, L/min/m^2	7.3 ± 0.1	4.5 ± 0.1[a]

Values are mean ± SEM or n/n. [a]$P < 0.001$ vs healthy subjects. [b]$P < 0.01$. [c]$P < 0.05$.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; BMI = body mass index; DBP = diastolic blood pressure; dPAP = diastolic pulmonary artery pressure; Ex = exercise; HFpEF = heart failure with preserved ejection fraction; HR = heart rate; LA = left atrial; LV = left ventricular; LVEDVI = left ventricular end-diastolic index; LVEF = left ventricular ejection fraction; MAP = mean arterial pressure; mPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; RAP = right arterial pressure; SBP=systolic blood pressure; sPAP = systolic pulmonary artery pressure.

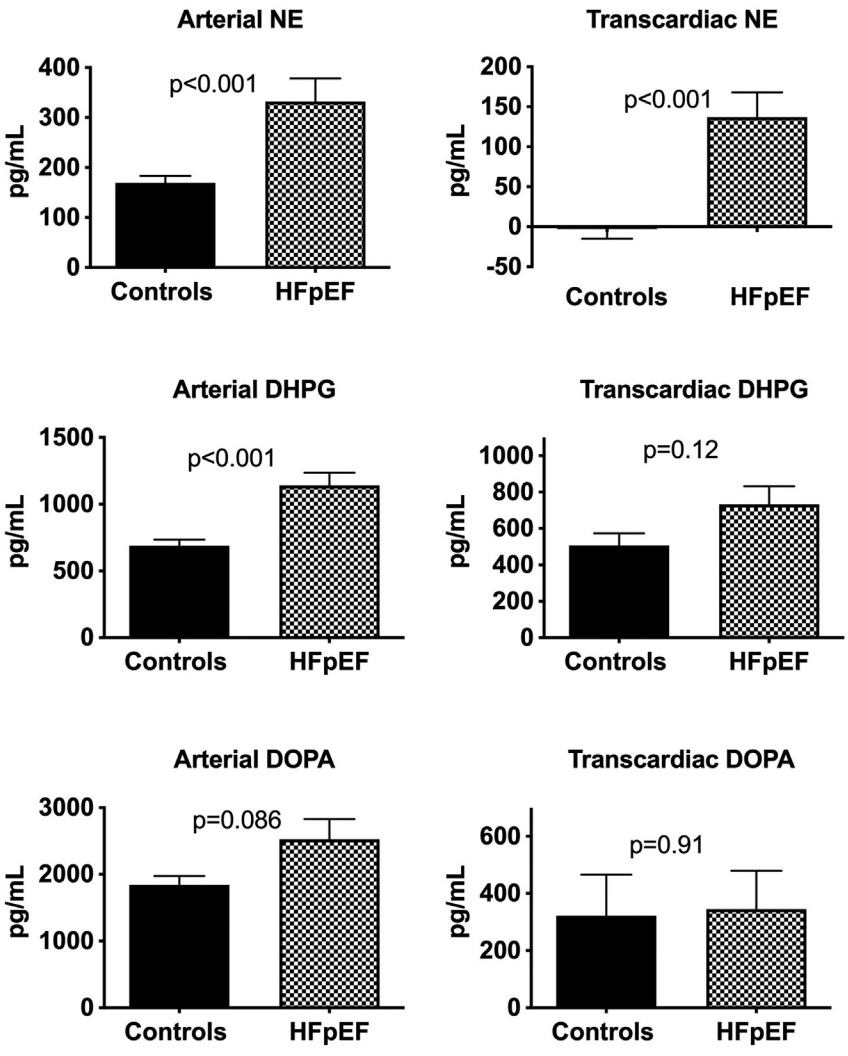
comparison to the healthy subjects (31 ± 1 vs 16 ± 1 mm Hg, $P < 0.001$).

SYSTEMIC AND CARDIAC SYMPATHETIC ACTIVITY IN HFpEF. As shown in Figure 1, HFpEF patients displayed evidence of systemic sympathoexcitation as evidenced by an increase in the plasma arterial norepinephrine (NE) concentration compared to healthy subjects (331 ± 36 pg/mL vs 169 ± 14 pg/mL, $P < 0.001$). This finding was further supported by the presence of significantly greater plasma levels of dihydroxyphenylglycol (DHPG, a major intraneuronal metabolite of recaptured NE, $P < 0.001$) and a trend towards higher levels of dihydroxyphenylalanine (DOPA, the neuronal NE precursor) (Figure 1). To evaluate the pattern of cardiac sympathetic activity in HFpEF we determined the transcardiac concentration gradients for NE (TC_{NE}), DHPG, and DOPA. As shown in Figure 1, we identified evidence for significant activation of cardiac sympathetic nerves as reflected by a marked increase in the TC_{NE} gradient. This finding was associated with a trend towards increased cardiac DHPG release, whereas the transcardiac DOPA gradient did not differ between groups.

We next investigated the potential hemodynamic and demographic drivers for cardiac and sympathetic activation. As shown in Figure 2, the TC_{NE} gradient was significantly correlated with the PCWP (r = 0.46, $P = 0.007$) and the arterial SBP (r = 0.47, $P = 0.006$). Given the close correlation between PCWP and mean PAP (r = 0.85, $P < 0.001$), we also identified a significant correlation between the TC_{NE} gradient and mean PAP (r = 0.46, $P = 0.009$), although this was numerically less than that with the PCWP. We also found a stronger correlation between the TC_{NE} gradient (measured at rest) and the exercise PCWP (r = 0.53, $P = 0.002$). The plasma arterial NE concentration was also significantly correlated with PCWP (r = 0.51, $P = 0.003$) and the SBP (r = 0.37, $P = 0.036$). In addition to the hemodynamic factors, the TC_{NE} gradient and arterial NE concentration were significantly correlated with age (r = 0.51, $P = 0.003$ and r = 0.61, $P < 0.001$, respectively), whereas neither the TC_{NE} gradient or arterial NE concentration were related to BMI (r = 0.29, $P = 0.11$ and r = 0.07, $P = 0.70$, respectively). Given the potential contribution of hemodynamic and demographic features that coexist with HFpEF, we conducted a multivariate analysis incorporating PCWP, SBP, age, BMI, and sex. Analysis of the determinants of the peripheral arterial NE plasma concentration identified only the PCWP as a significant contributor ($F_{1,24} = 6.01$, $P = 0.022$). In a similar analysis of the determinants of the TC_{NE} gradient, we identified SBP ($F_{1,24} = 5.55$, $P = 0.027$), together with a borderline association with PCWP ($F_{1,24} = 3.73$, $P = 0.065$). There were no differences between males and females. Finally we investigated the effect of cardiac rhythm on sympathetic activity. HFpEF patients in atrial fibrillation (AF) compared to those in sinus rhythm (SR) had significantly elevated arterial NE levels (AF vs SR: 398 ± 56 pg/mL vs 238 ± 16 pg/mL, $P = 0.019$) and a significantly elevated TC_{NE} gradient (AF vs SR: 192 ± 48 pg/mL vs 61 ± 23 pg/mL, $P = 0.036$). Furthermore, the arterial NE plasma levels and TC_{NE} gradients of HFpEF patients in SR was also significantly greater than that in healthy subjects ($P = 0.014$ and $P = 0.006$, respectively).

SYSTEMIC AND MYOCARDIAL INFLAMMATION IN HFpEF. We measured the arterial and coronary sinus plasma concentrations of 92 biomarkers of inflammation using the Olink proteomic platform. For 16 biomarkers, plasma concentrations were below the

FIGURE 1 Systemic and Cardiac Sympathetic Activity in HFpEF

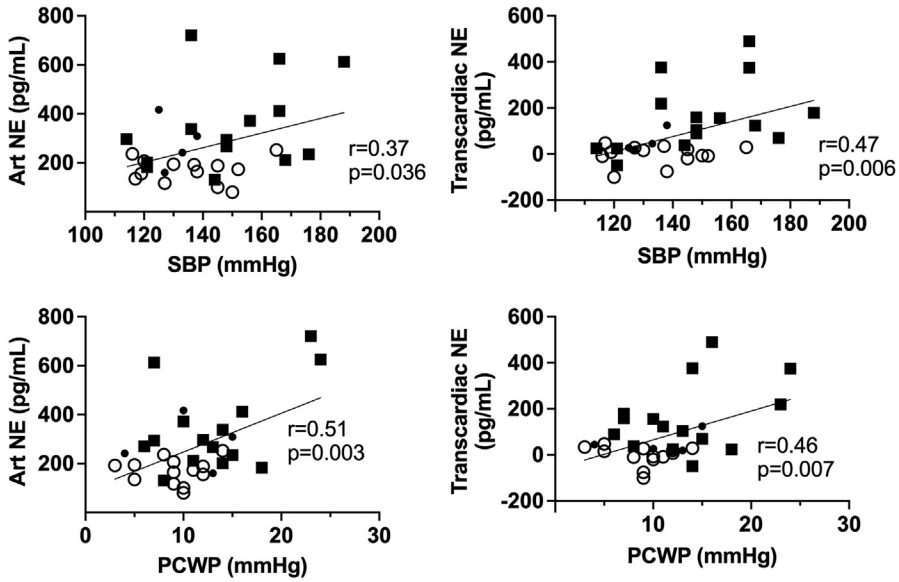


Bar graphs showing the plasma arterial concentration of norepinephrine (NE) and the transcardiac NE plasma concentration gradient and related metabolites dihydroxyphenylglycol (DHPG) and dihydyroxyphenylalanine (DOPA) in healthy subjects and heart failure with preserved ejection fraction (HFpEF) patients.

level of detection of the assay in several patients; therefore, these were removed from the analysis, leaving a total of 76 biomarkers. As shown in Figure 3A, healthy subjects and HFpEF patients showed a significantly ($P < 0.001$) different pattern of inflammatory biomarker expression in arterial blood as determined by PCA. By contrast, the transcardiac gradient of the inflammatory biomarkers did not differ between healthy subjects and HFpEF (Figure 3B).

As shown in Table 2, a total of 28 biomarkers of inflammation were demonstrated to be significantly elevated in the arterial plasma of HFpEF patients. By contrast, none of the transcardiac gradients of the inflammatory biomarkers in HFpEF patients were significantly different from that in healthy subjects. The magnitude of the average percentage change in the transcardiac gradient for the inflammatory markers was small (healthy subjects vs HFpEF: $-9 \pm 10\%$ vs $-2 \pm 2\%$, $P = 0.41$). In an exploratory analysis, we investigated potential demographic, autonomic, and hemodynamic drivers of elevated arterial cytokines in HFpEF. Specifically, we selected those cytokines in which the arterial plasma levels were at least 50% greater than in healthy subjects and included interleukin (IL)-17 (73%), fibroblast growth

FIGURE 2 Physiologic Correlates of Sympathetic Activity in HFpEF



Scatterplots showing the correlations between hemodynamic parameters and plasma arterial concentration of NE and the transcardiac NE plasma concentration gradient healthy subjects and HFpEF patients. PCWP= pulmonary capillary wedge pressure; other abbreviations as in Figure 1.

factor (FGF)-5 (71%), IL-6 (55%), and FGF-23 (53%). As shown in Table 3, we identified a significant correlation between age and the levels of IL-6, IL-17, FGF-23, and FGF-5. IL-6 levels were also correlated with PCWP and the arterial NE concentration. BMI was only correlated with IL-6 levels from the cytokines. FGF-5 levels were higher in females when combining the study groups (0.46 ± 0.15 vs 0.33 ± 0.13, $P = 0.037$); however, in multivariable analysis combining all factors, this difference no longer persisted. Given the multiple univariate correlates of plasma IL-6 levels, we further conducted a multivariable analysis. In this analysis, PCWP was the only significant determinant of plasma IL-6 levels ($F_{1,24} = 6.28$, $P = 0.006$).

DISCUSSION

In the present study, we have shown that HFpEF is characterized by both cardiac and systemic sympathetic activation, together with peripheral but not myocardial inflammatory activation. Our cohort of HFpEF patients were characterized by invasive exercise hemodynamics, which is considered to be the gold standard.[10] These data highlight the fact that peripheral measures of a particular biological process cannot necessarily be construed as representing the activity of their respective pathways within the myocardium. It is possible that very low levels of cytokine release from the myocardium below the limits of detection occurred, although the biological relevance of this would be uncertain. As such, our data provide new insights into the mechanisms that contribute to the myocardial pathogenesis of HFpEF. The relative paucity of studies that have directly characterized the myocardial biology of HFpEF by tissue or coronary sinus blood sampling was recently highlighted in an HFpEF taskforce report.[2]

The clinical features of HFpEF represent the net influences a complex set of physiologic abnormalities, both cardiac and extracardiac. Of paramount importance, measures of increased left atrial pressure, particularly during physical activity, are directly correlated with symptoms and outcomes, including cardiovascular mortality.[15,16] The mechanisms responsible for elevated filling pressures include increases in passive ventricular and atrial stiffness, impaired active ventricular relaxation that are further accentuated by the impact of increased arterial stiffness, and a failure of exercise-mediated vasodilatation.[1,11,17,18] To date, clinical trials of a multitude of interventions have not proven to significantly alter outcomes in HFpEF, suggesting that these treatments are not directed towards relevant cellular or physiologic targets. For example, it has been shown

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 7, NO. 2, 2022
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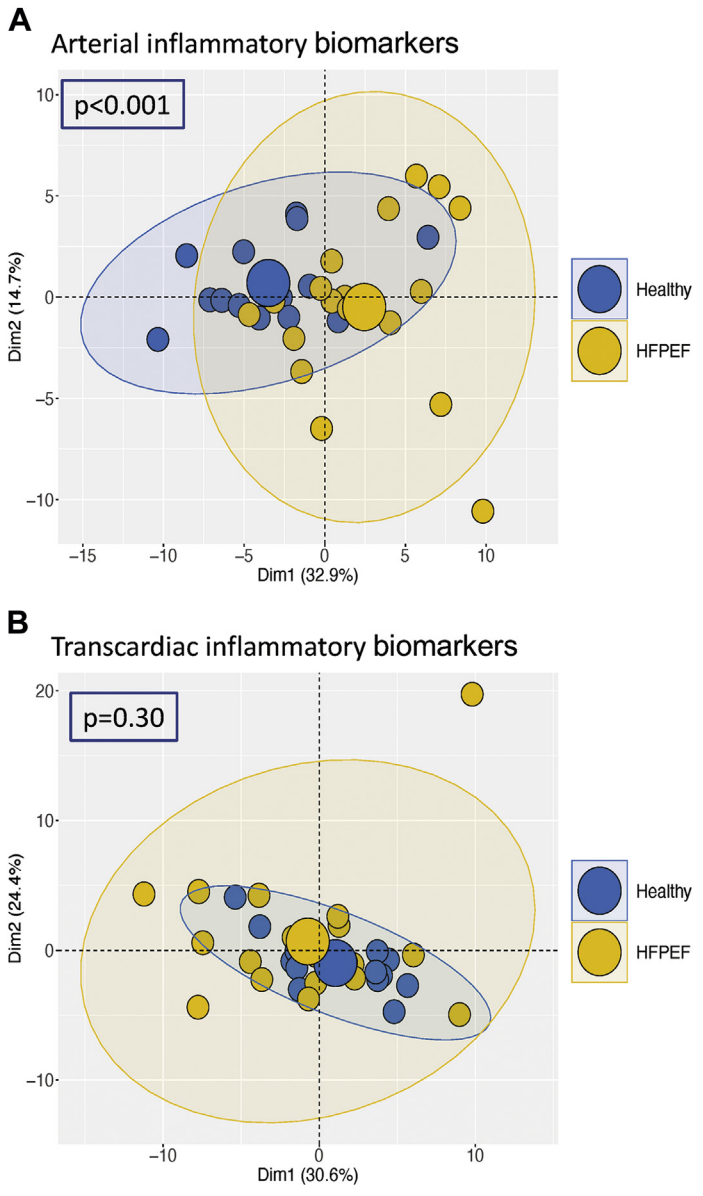
FIGURE 3 Systemic and Myocardial Cytokine Profiles in HFpEF



(A,B) Principal component analysis plots of the profile of plasma arterial concentrations of inflammatory cytokines and of their transcardiac concentration gradients in healthy subjects and HFpEF patients.

that the plasma levels of aldosterone, brain natriuretic peptide, and plasma renin activity are commonly within the normal range in patients with HFpEF, potentially explaining the limited impact of interventions directed towards these pathways.[19,20]

Our study contributes important new data regarding cardiac sympathetic activity in HFpEF patients, and its potential contribution to disease pathophysiology. Sympathetic neural drive shows substantial regional heterogeneity, limiting the ability of peripheral measures of sympathetic activity to reflect organ sympathetic tone.[21] This underscores the importance of our assessment of cardiac sympathetic drive. Activation of the sympathetic nervous system has been well demonstrated in HFrEF and hypertension, and in both cases the magnitude of sympathoexcitation has been correlated with clinical outcomes.[22-24] In this study we measured, for the first time, the net TC_{NE} in HFpEF patients and demonstrated it to be significantly greater than that in healthy subjects, signifying the presence of cardiac sympathetic activation. It is important to appreciate that the net TC_{NE} reflects the balance between local release from the sympathetic synaptic cleft to plasma, local uptake-mediated NE reuptake and flow.[21] Although in the current study we did not measure coronary sinus blood flow, it has been previously shown that resting myocardial blood flow is greater in HFpEF.[25] As such, it is unlikely that differences in myocardial blood flow account for the increased TC_{NE} in HFpEF patients. Similarly, our results cannot be explained by impaired reuptake of NE from sympathetic nerve endings. The current study revealed somewhat higher levels of DHPG, the intraneuronal metabolite of NE. It has been previously shown by our group that impaired reuptake of NE is associated with a decreased transcardiac DHPG gradient.[26] In the present study, transcardiac DOPA levels did not differ between healthy subjects and HFpEF. Although previous studies in HFrEF patients have indicated net release of DOPA from the heart, the net release of DOPA from sympathetic neurons is estimated to be small, suggesting that net cardiac DOPA release is only likely to be detected at high levels of neuronal NE synthesis.[26,27]

Our finding of elevated cardiac sympathetic drive raises 2 key questions. 1) What is the trigger for elevated cardiac sympathetic drive in HFpEF patients? 2) What are the consequences? In univariate analysis we found significant correlations between the TC_{NE} gradient and PCWP, age, and SBP. In a multivariable analysis, the association with SBP remained significant, with a borderline relationship with PCWP, although this analysis was likely influenced by the limited sample size. Of importance, in this analysis the diagnosis of HFpEF per se was not an independent determinant of cardiac sympathetic tone, suggesting that relevant hemodynamic factors are key determinants of cardiac adrenergic drive in HFpEF. This relationship between the PCWP and cardiac NE gradient may be mechanistically explained by the presence of left atrial (LA) and pulmonary venous stretch receptors which are

known to reflexively activate cardiac sympathetic nerves.[28] Consistent with this hypothesis, we have previously shown that cardiopulmonary baroreceptor unloading reduces cardiac adrenergic drive in HFrEF patients.[29] In this context, a pathophysiologic hallmark of HFpEF is the rapid increase in LA pressure during physical activity.[11,30] We also observed increased cardiac and systemic adrenergic drive in HFpEF patients in AF compared to those in SR; however, HFpEF patients in SR also exhibited greater sympathetic activity than the healthy volunteers. Whether the relationship between LA pressure and cardiac adrenergic activity drives a more marked exercise-mediated increase in cardiac sympathetic tone in HFpEF patients is not known. Of clinical relevance, increased cardiac sympathetic tone might be expected to contribute to the development of atrial arrhythmias.[31] In a similar context, the role of excess cardiac sympathetic drive as a trigger for ventricular arrhythmias is also well known, and it is recognized that sudden cardiac death contributes to a significant proportion of the cardiovascular deaths in HFpEF patients.[32,33] The complex interrelationship between SBP and cardiac sympathetic activity is consistent with previous studies in which we showed that cardiac sympathetic drive was increased in hypertensives with left ventricular hypertrophy (LVH) but not in those without LVH despite similar blood pressure.[34] We also found that the correlation between peak exercise PCWP and the TC_{NE} gradient was numerically greater than that at rest. This suggests that the level of cardiac sympathetic tone might be set by an integrated hemodynamic input in conjunction with acute hemodynamic stimuli.

In the present study we have also shown that the peripheral plasma NE concentration was increased in HFpEF patients compared with healthy subjects. Previous studies have generally shown that plasma NE levels are modestly elevated in HFpEF, although this finding has not been uniform possibly due variation in subject inclusion definitions, as reviewed by Badrov *et al.*[35] In univariate analysis we found that SBP, PCWP, and age were all significantly associated with the arterial NE concentration; whereas, in multivariate analysis only, PCWP was the only significant correlate and, consistent with the analysis for TC_{NE} release, we did not find HFpEF diagnosis per se to be an independent driver of the plasma NE concentration. Keir et al[35] recently examined the influence of age and sex on muscle sympathetic nerve activity (MSNA). These investigators found that MSNA increased with age in a nonlinear manner, with marked differences when comparing subjects aged 30 years versus 70 years. Similarly, our previous studies using radiotracer methodology showed that age may influence cardiac sympathetic tone when comparing a wide range of ages.[23] Keir *et al*[35] also evaluated the influence of sex, finding no difference in MSNA for those older than the age of 50 years, whereas significant differences were observed particularly in subjects aged 20 to 30 years.[36] Taken together, our data are consistent with the notion that many of the factors that are associated with HFpEF, including hemodynamic and demographic, lead to the autonomic phenotype observed in HFpEF. A much larger study with fully matched comorbidities in the absence of HFpEF symptoms would be required to fully

TABLE 2 Significantly Upregulated Cytokines in HFpEF

Cytokines	Healthy Subjects	HFpEF	q Value
FGF-23	2.48 (2.36-2.64)	3.56 (3.27-4.61)	<0.0001
CCL-20	5.31 (4.95-5.87)	6.36 (6.12-7.51)	<0.0001
IL-6	2.80 (2.19-3.23)	3.94 (3.62-4.60)	<0.0001
CXCL-10	9.08 (8.68-9.67)	10.37 (9.91-10.69)	<0.0001
FGF-5	0.21 (0.19-0.35)	0.45 (0.39-0.54)	0.0001
DNER	9.01 (8.81-9.06)	8.65 (8.43-8.80)	0.0001
VEGFA	9.51 (9.31-9.66)	9.95 (9.72-10.38)	0.0001
IL-17C	0.62 (0.56-0.86)	1.04 (0.94-1.48)	0.0003
CSF-1	9.82 (9.69-9.99)	10.08 (9.99-10.24)	0.0005
OPG	9.38 (9.09-9.57)	9.77 (9.58-9.97)	0.0005
IL-8	5.25 (4.56-5.57)	6.08 (5.51-6.32)	0.0006
CDCP1	3.50 (3.32-3.97)	4.21 (3.92-4.65)	0.0007
Beta-NGF	0.86 (0.78-0.96)	1.05 (1.01-1.18)	0.0007
CXCL9	6.83 (6.05-7.31)	7.59 (7.01-8.46)	0.0010
PD-L1	6.43 (6.15-6.59)	6.97 (6.68-7.27)	0.0011
HGF	8.45 (8.16-8.76)	8.94 (8.65-9.23)	0.0011
MCP-3	1.01 (0.73-1.57)	1.77 (1.32-2.09)	0.0021
CXCL-11	7.34 (7.15-8.07)	8.27 (7.89-9.10)	0.0023
MCP-4	12.91 (12.68-13.33)	13.61 (13.29-14.26)	0.0026
CCL-19	8.78 (8.67-9.24)	9.37 (9.01-9.86)	0.0037
CCL-3	5.06 (4.77-5.53)	5.88 (5.35-6.36)	0.0063
CD40	11.26 (10.88-11.49)	12.06 (11.44-12.40)	0.0078
CCL-11	6.96 (6.72-7.09)	7.35 (7.05-7.77)	0.0087
TNFRSF9	6.26 (6.09-6.55)	6.63 (6.38-7.00)	0.0096
TGF-α	3.84 (3.70-3.97)	4.00 (3.94-4.26)	0.0118
IL-12B	5.85 (5.25-6.21)	6.37 (5.86-6.67)	0.0130
Flt3L	8.79 (8.69-9.13)	9.19 (8.87-9.55)	0.0158
LIF-R	3.09 (2.92-3.23)	3.29 (3.08-3.45)	0.0173

Values are median with 25th and 75th percentiles (Q1-Q3).

Beta-NGF = beta nerve growth factor; CCL = C-C motif chemokine; CD40 = cluster of differentiation 40; CDCP1 = CUB domain containing protein 1; CSF = colony stimulating factor; CXCL = C-X-C motif chemokine; DNER = delta and notch-like epidermal growth factor related receptor; FGF = fibroblast growth factor; Flt3L = FMS-like tyrosine kinase 3 ligand; HGF = hepatocyte growth factor; IL = interleukin; LIF-R = leukemia inhibitory factor receptor; MCP = monocyte chemotactic protein; OPG = osteoprotegerin; PD-L1 = programmed cell death 1 ligand 1; TGF = transforming growth factor; TNFRSF9 = tumor necrosis factor receptor superfamily member 9; VEGFA = vascular endothelial growth factor A.

TABLE 3 Hemodynamic and Demographic Correlates of Systemic Inflammation

	IL-17		FGF5		IL-6		FGF23	
	r	*P* Value	r	*P* Value	r	*P* Value	r	*P* Value
BMI	-0.01	0.98	0.22	0.22	**0.45**	**0.007**	0.25	0.15
SBP	0.27	0.12	0.22	0.22	0.18	0.92	-0.06	0.74
Age	**0.39**	**0.021**	**0.47**	**0.005**	**0.46**	**0.006**	***0.64***	**<0.001**
PCWP	-0.03	0.86	0.16	0.35	**0.53**	**0.001**	**0.42**	**0.012**
Arterial plasma NE	0.28	0.13	0.32	0.08	**0.50**	**0.004**	0.24	0.20

Statistically significant correlations are in **bold**.
Abbreviations as in Table 1.

elucidate the contribution of each potential factor. Given the invasive nature of our study, this would be particularly challenging.

The use of antiadrenergic therapies in HFpEF remains controversial, and, in particular, the role of beta blockade is uncertain. Although observational studies have suggested potential benefit, this has not been supported by randomized trials.[37] The relative importance of myocardial beta receptors in the pathophysiology of HFpEF is unclear. By contrast, alpha receptors have been implicated in that pathogenesis of cardiac fibrosis in the context of excess sympathetic drive.[38] The utility of centrally acting sympathoinhibitory drugs has not been investigated in hypertensive HFpEF patients.

Elevated peripheral levels of several inflammatory cytokines, including members of the IL, tumor necrosis factor, and chemokine families are well-described in HFpEF, consistent with the present findings.[6,39] A key finding of our study is that the myocardium does not appear to release cytokines in HFpEF. As such, these data provide further insights into origins of inflammation in HFpEF and into its potential role in the pathophysiology of HFpEF.

In the present study we showed a positive correlation between age with several peripheral biomarkers of inflammation including IL-6, IL-17, FGF-5, and FGF-23. Our finding of a 54% increase in plasma IL-6 levels in HFpEF patients is consistent with prior studies in HFpEF patients.[39,40] Although the healthy subjects were significantly younger than HFpEF patients, the difference in IL-6 levels could not be explained on the basis of age alone in a multivariable analysis. The relationship between aging and inflammation is well recognized; however, the underpinning mechanism(s) remain speculative.[41] Proposed contributing mechanisms for the process of "inflammageing" include visceral obesity, gut dysbiosis, chronic infection, and altered immune regulation.[41] In addition to age per se, many of the putative contributors cluster in patients with HFpEF. For example, we have recently shown the presence of gut dysbiosis in HFpEF patients and epidemiologic data clearly indicate the close association between obesity the prevalence of HFpEF.[42,43] In the current study, we found a close relationship between BMI and IL-6 levels, and expression of IL-6 in adipose tissue has been shown to be increased at the mRNA and protein level in obesity.[44,45] It has been shown that the abundance of intra-abdominal visceral adipose tissue is strongly predictive of HFpEF risk, leading to speculation that activation of inflammatory pathways contributes to the pathogenesis of HFpEF rather than as an epiphenomenon.[46-48] Consistent with this, patients with nonmetabolic, noncardiovascular causes of inflammation such as rheumatologic disorders also have evidence of diastolic dysfunction consistent with the notion that inflammatory cytokines may, in part, drive the development of HFpEF.[48] Interestingly, visceral adiposity is also associated with sympathetic activation, as we also observed in HFpEF patients.[49]

From a mechanistic perspective, IL-6 is a cytokine with a pleiotropic repertoire of actions, including stimulation of fibroblast proliferation and the biosynthesis of extracellular matrix proteins.[50,51] Genetic deletion of IL-6 attenuated myocardial fibrosis in hypertensive mice secondary to angiotensin II/high salt.[52] IL-6 signaling is complex and involves its binding to a membrane signaling complex that comprises an IL-6 receptor and the gp130 transmembrane protein. This mechanism is negatively regulated by a circulating complex formed by IL-6 together soluble fractions of gp130 and IL-6R.[53] Whether the increased circulating levels of IL-6 are definitively pathogenic in HFpEF is unknown. However, the plasma

concentrations observed in HFpEF patients have been shown to increase expression of matrix metalloproteinase (MMP)-2 in cell culture studies.[54]

In addition to aging and obesity as likely drivers of inflammation, we observed significant correlations between the peripheral plasma levels of IL-6 and FGF-23 with PCWP. This observation raises the possibility that the lung may release cytokines in HFpEF under the influence of elevated filling pressures. This observation is consistent with recent studies of pulmonary cytokine expression in experimental HF.[55] It is also possible that a more complex balance between hemodynamics, obesity, and inflammation is present as suggested recently by Sorimachi *et al*.[56] We observed elevated levels of FGF-23 in HFpEF patients, and the plasma levels of FGF-23 were found to correlate significantly with PCWP. FGF-23 has previously been shown to be associated with increased risk of cardiovascular disease, pulmonary hypertension, LVH, AF, and mortality.[57-59] This finding suggests the concept that the lung itself might be an important source of FGF-23 under the influence of elevated pulmonary pressures.

In the current study, we did not detect any evidence of inflammatory cytokine release from the myocardium in HFpEF patients. Recently it has been proposed that epicardial adipose tissue (EAT) may play a role in the pathophysiology of HFpEF either by a direct mechanical effect or by the release of inflammatory cytokines in a manner similar to visceral adipose tissue.[60] Previously, it has been shown that levels of expression of inflammatory cytokines including IL1-b, IL-6, and tumor necrosis factor (TNF)-α at the mRNA and protein level are elevated in EAT compared to peripheral subcutaneous fat.[61] However, these studies did not investigate the levels of inflammatory cytokines within the myocardium per se. Although the presence of metabolically active EAT in apposition with epicardial coronary arteries acting as an amplifier of vascular inflammation is widely accepted, the influence of EAT as a direct paracrine source of cytokines which drive myocardial remodeling in HFpEF is more speculative. Our findings are consistent with prior studies performed in patients with coronary disease in which no transcardiac gradient of IL-6 and TNF-α was detectable.[62-64] Given that the myocardium and EAT share a common microcirculation, we would have predicted that a significant transmyocardial gradient would be detectable in the presence of significant release of cytokines by the EAT.[65] We did not assess the volume of EAT in this study; however, our HFpEF patient profiles are consistent with other studies in which increased EAT volumes have been measured in HFpEF patients.[66] Nevertheless, we cannot exclude the presence of a small concentration of gradients that lie beneath the sensitivity of the aptamer assay, although previous studies used an enzyme-linked immunosorbent assay–based assay. Similarly, we did not obtain myocardial biopsy specimens. Although these studies have shown the presence of inflammatory cells such as CD68 cells, the level of increase above that seen in healthy subjects is only modest.[67] We cannot exclude the possibility that cytokines released by EAT are drained by myocardial and pericardial lymphatics, although the myocardial lymph flow rate is relatively small.[68]

STUDY LIMITATIONS. The sample size in this study was relatively small; however, we conducted a detailed invasive study which included healthy normal volunteers. Despite the small sample size, we were able to detect evidence of cardiac sympathetic activation and systemic inflammation compared to the healthy volunteers. We assayed a large number of cytokines and used a PCA-based approach to reduce the dimensionality of the data. Our between-group comparisons were appropriately statistically corrected for the number of between-group comparisons. As observed in the current study, HFpEF patients are characterized by key hemodynamic features in conjunction with a cluster of comorbidities including aging, hypertension, obesity, AF, diabetes, and female sex which may all drive both systemic and cardiac sympathetic activity and systemic inflammation. It is likely that the pathophysiology of HFpEF results from the combined influence of all of these factors to a certain extent. Because of the challenging nature of recruiting healthy volunteers for invasive studies, we were not able to match subjects appropriately with HFpEF patients to elucidate the contribution of each contributing comorbidity individually.

CONCLUSIONS

The current study provides new insights into the complex nature of HFpEF pathophysiology. In particular, we showed the presence of elevated cardiac sympathetic activity in HFpEF patients and extend prior evidence supporting the presence of systemic sympathetic and inflammatory activation. By contrast, we did not find evidence in support of inflammatory activation in the myocardium of HFpEF patients as evaluated by transcardiac cytokine gradients. Our

findings may offer potential insights into mechanisms that might underpin the recently reported positive effects of sodium-glucose transport protein 2 inhibition in HFpEF patients.[5] The current findings are likely to reflect the integrated effects of the typical cluster of comorbidities that associate with HFpEF, acting together with the characteristic hemodynamic profile. Finally, the finding of cardiac sympathetic activation in HFpEF patients requires further investigation as a potential contributor to cardiovascular outcomes and therefore it may be a therapeutic target.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This work was supported by a National Health & Medical Research Council (NHMRC) of Australia Fellowship support to Dr Kaye. Dr Marques is supported by a National Heart Foundation Future Leader Fellowship and National Heart Foundation Grants. The Baker Heart & Diabetes Institute is supported in part by the Victorian Government's Operational Infrastructure Support Program. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Dr David M. Kaye, Heart Failure Research Group, Baker Heart and Diabetes Institute, PO Box 6492, St Kilda Road Central, Melbourne, Victoria 8008, Australia. E-mail: david.kaye@baker.edu.au.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Advances in the management of HF, particularly HFrEF, are the result of detailed mechanistic studies. Although HFpEF accounts for approximately 50% of all HF cases, limited human data is available regarding local myocardial pathophysiology. The current study shows that HFpEF is characterized by activation of the cardiac sympathetic nervous system; however, we did not detect evidence for local myocardial inflammation. Our findings suggest that a re-evaluation of the role of antiadrenergic therapies may be warranted in HFpEF patients.

TRANSLATIONAL OUTLOOK: Left ventricular remodeling is a fundamental feature of HF. The local pathophysiological inputs that drive this process in HFpEF have not been investigated in detail. Coordinated investigation of the autonomic, inflammatory, and metabolic provide of the myocardium in HFpEF patients will allow the identification and manipulation of therapeutic targets that can subsequently be translated into clinical trials.

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KEY WORDS **cytokines, heart failure with preserved ejection fraction, sympathetic nervous system, transcardiac biomarker gradient**