Plasma Biomarkers Reflecting Profibrotic Processes in Heart Failure With a Preserved Ejection Fraction

Data From the Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction Study

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Background—Heart failure with preserved ejection fraction is a clinical syndrome that has been associated with changes in the extracellular matrix. The purpose of this study was to determine whether profibrotic biomarkers accurately reflect the presence and severity of disease and underlying pathophysiology and modify response to therapy in patients with heart failure with preserved ejection fraction.

Methods and Results—Four biomarkers, soluble form of ST2 (an interleukin-1 receptor family member), galectin-3, matrix metalloproteinase-2, and collagen III N-terminal propeptide were measured in the Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction (PARAMOUNT) trial at baseline, 12 and 36 weeks after randomization to valsartan or LCZ696. We examined the relationship between baseline biomarkers, demographic and echocardiographic characteristics, change in primary (change in N-terminal pro B-type natriuretic peptide) and secondary (change in left atrial volume) end points. The median (interquartile range) value for soluble form of ST2 (33 [24.6–48.1] ng/mL) and galectin 3 (17.8 [14.1–22.8] ng/mL) were higher, and for matrix metalloproteinase-2 (188 [155.5–230.6] ng/mL) lower, than in previously published referent controls; collagen III N-terminal propeptide (5.6 [4.3–6.9] ng/mL) was similar to referent control values. All 4 biomarkers correlated with severity of disease as indicated by N-terminal pro B-type natriuretic peptide, E/E′, and left atrial volume. Baseline biomarkers did not modify the response to LCZ696 for lowering N-terminal pro B-type natriuretic peptide; however, left atrial volume reduction varied by baseline level of soluble form of ST2 and galectin 3; patients with values less than the observed median (<33 ng/mL soluble form of ST2 and <17.8 ng/mL galectin 3) had reduction in left atrial volume, those above median did not. Although LCZ696 reduced N-terminal pro B-type natriuretic peptide, levels of the other 4 biomarkers were not affected over time.

Conclusions—In patients with heart failure with preserved ejection fraction, biomarkers that reflect collagen homeostasis correlated with the presence and severity of disease and underlying pathophysiology, and may modify the structural response to treatment.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00887588.

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Several novel circulating biomarkers have been used to characterize the molecular and cellular changes that occur during the development of myocardial disease.[1–4] In heart failure (HF), these include biomarkers that reflect hemodynamic status (such as natriuretic peptides), inflammation (such as interleukins), and collagen homeostasis (such as collagen peptides and interstitial proteases).[2] The profiles of these biomarkers seem to differ significantly in patients with HF with a reduced ejection fraction (EF) versus HF with a preserved EF (HFpEF.[5–7] The nature and extent to which biomarkers change in HFpEF as a function of disease severity, degree of left ventricular (LV) structural and functional abnormalities and demographics and comorbid conditions have not been fully defined. In addition, whether baseline biomarkers can modify the response to treatment in HFpEF has not been examined. One potential mechanism hypothesized to play a pivotal role in the development of HFpEF is a change in collagen homeostasis that results in extracellular matrix fibrosis and the development of abnormal diastolic function.[8–12] Several molecular and cellular signaling pathways that result in a profibrotic milieu have been identified in previous studies but have not been specifically examined in randomized clinical trials in HFpEF.[2,6,7]

See Clinical Perspective

Patients enrolled in the Prospective Comparison of ARNI With ARB on Management of Heart Failure with Preserved Ejection Fraction trial (PARAMOUNT) had the clinical syndrome of heart failure and evidence of increased LV filling pressures (symptoms and signs of volume overload and increased N-terminal pro B-type natriuretic peptide [NT-proBNP]).[13] We hypothesized that if changes in extracellular matrix fibrillar collagen content lead to abnormal diastolic function, these patients should have changes in biomarkers reflecting this. The purpose of this study was to examine a selected portfolio of postulated profibrotic biomarkers in a defined population of HFpEF, relate these biomarkers to demographic characteristics, changes in LV structure and function, severity of disease and response to treatment. Given the post hoc nature of this analysis and its modest sample size, this study was envisioned as hypothesis generating providing provocative evidence that would lead to additional, larger, and more definitive studies.

Methods

Study Design

PARAMOUNT was a randomized, double-blind, parallel group, active control trial described in detail in previous publications.[13] Briefly, men and women aged ≥40 years old with an LVEF ≥45% and a documented history of heart failure with associated signs or symptoms (dyspnea on exertion, orthopnea, paroxysmal dyspnea, and peripheral edema) were eligible. Patients were required to have NT-proBNP >400 pg/mL at screening, be on diuretic therapy, and have a systolic blood pressure <140 or ≤160 mm Hg, or less if on ≥3 blood pressure drugs at randomization, have an estimated glomerular filtration rate (eGFR) of at least 30 mL/min per 1.73 m^2 at screening, and a potassium concentration of no >5.2 mmol/L. Patients were excluded if they had previous LVEF <45% at any time, isolated right heart failure because of pulmonary disease, dyspnea because of noncardiac causes, such as pulmonary disease, anemia, or severe obesity, primary valvular or myocardial diseases, or coronary artery or cerebrovascular disease needing revascularization within 3 months of screening or likely to need revascularization during the trial. The number of patients enrolled with atrial fibrillation on ECG at screening was limited to roughly 25% of the total. The study protocol was submitted to individual sites' institutional review boards or ethics committees and all enrolled patients provided written informed consent. A data safety monitoring committee oversaw the program and reviewed trial data for patient safety at regular intervals.

Biomarkers

Plasma and serum were obtained for biomarker determination at baseline, 12, and 36 weeks after randomization. The baseline measurements were made at randomization after a placebo run-in phase. Measurements after randomization to valsartan or LCZ696 were made in 12 and 36 weeks. NT-proBNP and matrix metalloproteinase-2 (MMP-2) were measured in plasma and collagen III N-terminal propeptide (PIIINP) was measured in serum at Quest Diagnostics (Valencia, CA) using the Elecsys proBNP immunoassay (Roche Diagnostics, Indianapolis, IN), the Quantikine MMP-2 immunoassay (R and D Systems, Minneapolis, MN), and the UniQ PIIINP radioimmunoassay (Orion Diagnostics, Espoo, Finland). The soluble form of ST-2 (sST-2, an interleukin-1 receptor family member) was measured in serum at Critical Diagnostics (San Diego, CA) using their Presage immunoassay, and Galectin 3 (Gal-3) was measured in serum at Clinical Reference Laboratories (Lenexa, KS) using an enzyme-linked immunosorbent assay (BG Medicine, Waltham, MA).

Echocardiographic Study

Baseline echocardiograms were analyzed in the cardiovascular imaging core laboratory at Brigham and Women's Hospital, Boston, MA. All measurements were made in triplicate in accordance with the recommendations of the American Society of Echocardiography.[14] LV volumes, mass, relative wall thickness, mitral flow velocities, tissue Doppler velocities, LAV, and LVEF were calculated according to the recommendations of the American Society of Echocardiography.[14] Left atrial strain and LV global longitudinal strain were measured using vendor-independent 2-dimensional speckle tracking software.[15]

Statistical Analysis

Baseline characteristics were summarized by quartiles of each biomarker using counts and percentages for binary variables and means and SDs for continuous variables, with the exception of NT-proBNP, which is summarized via median (interquartile range [IQR]) because of skewness. Tests for trend across quartiles were conducted via χ^2 trend tests, linear regression, and Cuzick nonparametric trend test, as appropriate. Biomarker data were presented as mean±SEM, geometric mean, and median (IQR) at baseline. Baseline biomarker data were compared with data measured at 12 and 36 weeks after randomization presented as median (IQR) for the entire study group and then divided according to treatment group (valsartan versus LCZ696).

Baseline biomarker data were compared qualitatively with referent control values. Referent control values were presented for comparison as median (IQR). Median (IQR) referent control data for PIIINP and MMP-2 were taken from a previously published study in which 241 subjects of age, sex, and race distribution similar to this study population were examined.[5] However, these well-characterized subjects had no clinical, serological, or cardiac structural/functional abnormalities as evidenced by a normal echocardiography and 6-minute hall walk distance. Median (IQR) referent control data for Gal-3 were taken from a previously published study in which 1092 subjects of age, sex, and race distribution similar to this study population were examined.[16] Median (IQR) referent control data for sST-2 were aggregated from 3 previously published study (including the Framingham study) in which subjects of age, sex, and race distribution similar to this study population were examined.[17–20] Although small differences between men and women have been seen in the biomarkers described above, because the populations of both this study and the referent control populations are roughly 50% female, the referent control values listed in Table 1 represent the total population

Table 1. Biomarker Data

	PARAMOUNT-HF HFpEF Patients			Referent Controls
	Mean (SD)	Geo Mean	Median (IQR)	Median*
sST2, ng/mL	39.6 (24.7)	34.7	33.0 (24.6–48.1)	20 (17–26)
Galectin-3, ng/mL	19.0 (6.9)	17.9	17.8 (14.1–22.8)	12 (9–15)
MMP-2, ng/mL	198 (73)	184	188 (156–231)	335 (323–443)
PIIINP, ng/mL	6.1 (3.5)	5.5	5.6 (4.3–6.9)	6.5 (6.1–8.2)

HFpEF indicates heart failure with preserved ejection fraction; IQR, interquartile range; MMPs, matrix metaloproteinases; PARAMOUNT, Prospective Comparison of ARNI With ARB on Management of Heart Failure with Preserved Ejection Fraction; PIIINP, collagen III N-terminal propeptide; and sST2, soluble form of ST2 (an Interleukin-1 receptor family member).

*Referent control median (IQR) values taken from the studies by Zile et al,[5] Motiwala et al,[25] Wang et al,[26] Bhardwaj et al,[27] and Shah et al.[28]

examined. In addition, biomarker data from this study of patients with HFpEF were compared with previously published groups of patients with HFpEF.[5,21–24] Finally, Gal-3 and sST-2 data in this study were compared with Food and Drug Administration approved partition values for risk stratification; these partition values were not specifically designed for risk stratification in HFpEF but were targeted to overall risk in generalized populations.

Correlations between biomarkers and demographic and echocardiographic data were performed using Spearman correlation. Values for sST-2, Gal-3, and NT-proBNP were log transformed because they were noticeably right skewed. In a multivariable regression model that included age, sex, New York Heart Association class, history of atrial fibrillation, diastolic blood pressure, eGFR, log NT-pro BNP, LV transmitral early diastolic filling velocity/LV early diastolic myocardial velocity (E/E′), and LA volume, we examined which factors were independently associated with baseline levels of biomarkers. Variables included were on the basis of low numbers of missing values and clinical knowledge. To examine the interaction between treatment with LCZ696 and baseline biomarker levels on levels of NT-proBNP at 12 weeks and LA volume at 36 weeks, we used a regression model that included the effect of LCZ696, an interaction term between treatment and baseline biomarker values and stratification variables of region and previous angiotensin-converting-enzyme inhibitor or ARB use as well as baseline NT-proBNP and LA volume, respectively. Where an interaction was found this was explored further by dividing the cohort into values above and below the observed median value of the biomarker and stratified models of the effect of treatment on NT-proBNP and LA volume examined. All statistical analyses were performed using STATA version 12 (Stata Corp, College Station, TX). For interaction tests a $P<0.1$ was considered suggestive of an interaction, and for all other test a $P<0.05$ was considered statistically significant.

Results

Demographic and Echocardiographic Data

Data from the 301 randomized patients were included in this study, 149 randomized to LCZ696 and 152 to valsartan. The demographic and echocardiographic data were typical of a stable outpatient HFpEF population[29,30]; elderly, female, and NYHA class II dominant, receiving multidrug treatment, expected comorbidities, and evidence of abnormal diastolic function with increased NT-proBNP, E/E′, and LA volume. Baseline data are presented for each individual biomarker examined by quartiles (Tables I–IV in the Data Supplement).

Baseline Biomarker Data

In these patients with HFpEF, the baseline median values for Gal-3 and sST-2, summarized in Table 1, were ≈50% higher than the median values from previously published referent control subjects.[16–24] In these patients with HFpEF, the median values for MMP-2 were ≈50% lower and PIIINP were similar to previously published referent control subjects.[5,31]

There were significant correlations between baseline biomarkers and demographic and echocardiographic variables (Table 2). Gal-3, sST-2, and PIIINP increased and MMP-2 decreased in association with an increase in NT-proBNP, and decreased eGFR (Figure 1A). sST-2 increased and MMP-2 decreased in association with an increase in E/E′ and LA volume (Figure 1B). There was also a direct relationship between sST-2 and Gal-3 ($r=0.24$, $P<0.001$; Figure 1C).

Table 2. Correlations Between Biomarker and Demographic/Echocardiographic Data

	ST2		Galectin		MMP2		PIIINP	
	R	*P*	*R*	*P*	*R*	*P*	*R*	*P*
NT-proBNP	0.19	0.002	0.17	0.004	0.31	<0.001	0.25	<0.001
eGFR	−0.16	0.005	−0.50	<0.001	−0.19	0.002	−0.14	0.07
SBP	−0.03	0.64	0.01	0.94	0.01	0.92	−0.04	0.60
E′	0.07	0.31	−0.04	0.51	0.07	0.31	0.07	0.38
E/A	0.12	0.11	−0.04	0.57	0.24	0.003	−0.02	0.84
E/E′	0.11	0.09	0.09	0.18	0.17	0.01	−0.03	0.70
LA volume	0.25	<0.001	−0.01	0.87	0.14	0.03	−0.04	0.65

A indicates atrial contraction induced diastolic filling velocity wave; E, early diastolic filling velocity; E′, early diastolic myocardial velocity; eGFR, estimated glomerular filtration rate; LA, left atrium; MMPs, matrix metaloproteinases, NT-proBNP, N-terminal pro-B-type natriuretic peptide; PIIINP, collagen III N-terminal propeptide; SBP, systolic blood pressure; and sST-2, soluble form of ST2 (an interleukin-1 receptor family member).

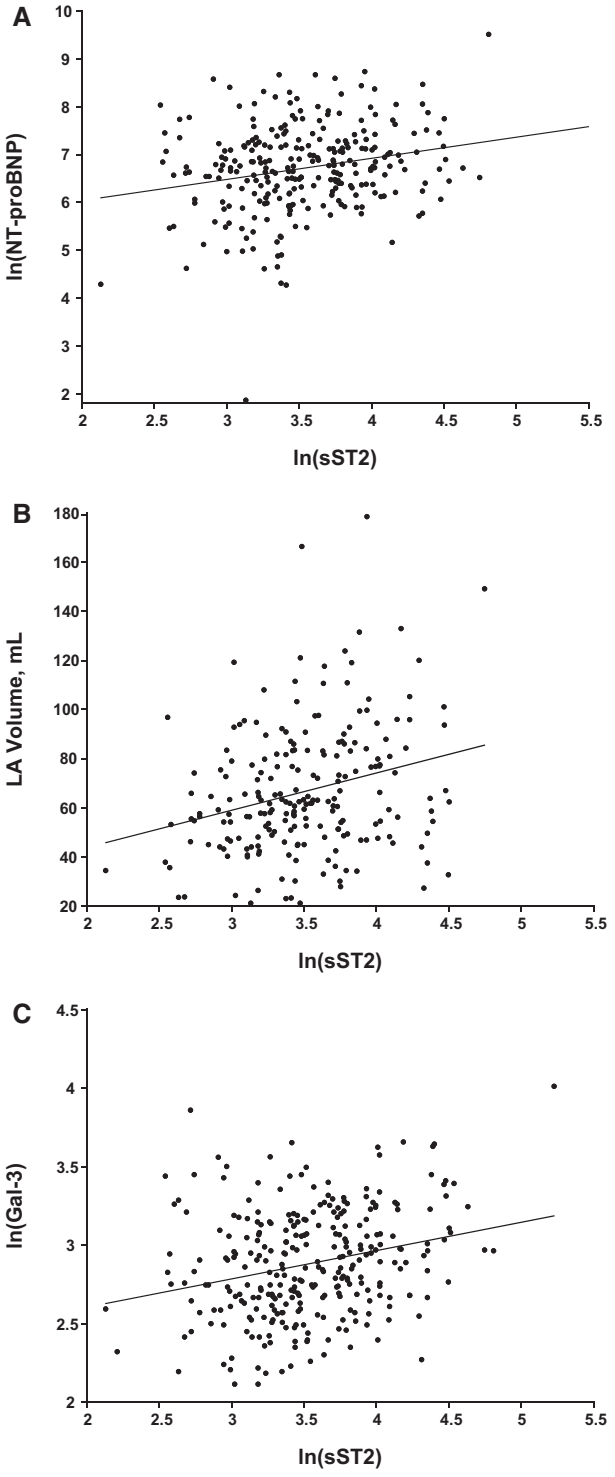

Figure 1. A, Relationship between ln(sST-2) and ln(NT-proBNP) in patients with heart failure with a preserved ejection fraction; Spearman correlation r=0.19, P=0.002. **B**, Relationship between ln(sST2) and left atrial volume (LAV) in patients with heart failure with a preserved ejection fraction; correlation r=0.25, P=0.002. **C**, Relationship between ln(sST2) and ln(Gal-3) in patients with heart failure with a preserved ejection fraction; correlation r=0.23, P<0.0001. ; Gal-3 indicates galectin 3; NT-proBNP, N-terminal pro B-type natriuretic peptide; and sST-2, soluble suppressor of tumorigenicity-2.

In a multivariable model after adjusting for age, sex, NYHA class, history of atrial fibrillation, diastolic blood pressure, eGFR, log NT-proBNP, E/E′, and LA volume, only female sex (coefficient, −0.27 [95% CI, −0.40 to −0.14]; P<0.001), NYHA class (coefficient, 0.24 [0.09 to 0.39]; P=0.002), and LA volume (coefficient, 0.003 [0.0004 to 0.005]; P=0.02) were statistically significantly associated with higher sST2. Only female sex (coefficient, 0.168 [0.004–0.332]; P=0.04) and log NT-proBNP (coefficient, 0.10 [0.01–0.19]; P=0.03) were associated with baseline PIIINP. Lower diastolic blood pressure was associated with higher MMP-2 levels at baseline (coefficient, −0.014 [−0.023 to −0.006]; P=0.001), whereas lower eGFR was associated with higher Gal-3 (coefficient, −0.008 [−0.011 to −0.006]; P<0.001).

Relationship Between Biomarkers and LCZ696 Treatment

The relationship between biomarkers and the effects of LCZ696 on the primary study end point (change in NT-proBNP after 12 weeks of treatment) and the secondary study end point (change LA volume from baseline after 36 weeks of treatment) were examined. There were no treatment interactions between LCZ696 and changes in NT-proBNP at 12 weeks (primary end point) for any of the 4 biomarkers: MMP-2 (interaction P=0.40), PIIINP (interaction P=0.14), Gal-3 (interaction P=0.32), or sST2 (interaction P=0.63). In addition, there were no treatment interactions between LCZ696 and changes in NT-proBNP at 36 weeks for any of the 4 biomarkers: MMP-2 (interaction P=0.09), PIIINP (interaction P=0.5), Gal-3 (interaction P=0.2), or sST2 (interaction P=0.99). However, there was an interaction between the response to treatment with LCZ696 compared with valsartan on change in LA volume at 36 weeks and baseline values of sST-2 (interaction P=0.07) and Gal-3 (interaction P=0.04). There was no interaction with PIIINP levels (interaction P=0.79) or MMP2 (interaction P=0.61).

We further explored the interaction with sST-2 and Gal-3 by dividing patients into 2 groups, for those above and below the observed baseline median value of sST-2 (≥33.0 versus <33.0 ng/mL) or Gal-3 (≥17.8 versus <17.8 ng/mL). The effect on change in LA volume from baseline differed between LCZ696 and valsartan (Figure 2). In patients with a baseline value of sST-2 above the median, 36 weeks of treatment with LCZ696 did not result in a significant change in LA volume from baseline compared with valsartan (difference, −1.5; 95% CI, −7.8 to 4.8; P=0.6). Similarly in patients with a baseline value of Gal-3 above the median, treatment with LCZ696 did not result in a significant change in LA volume from baseline compared with valsartan (difference, −1.8, 95% CI, −7.6 to 4.0; P=0.5). In patients with a baseline value of sST-2 or Gal-3 below the median, 36 weeks of treatment with LCZ696 resulted in a statistically significantly larger change from baseline in LA volume compared with valsartan (in those with sST-2 values below median, the difference in treatment effect between LCZ696 versus valsartan was −9.9; 95% CI, −15.1 to −4.8; P<0.0001, in those with Gal-3 values below the median treatment effect between LCZ696 versus valsartan was median difference −10.3; 95% CI, −15.6 to −5.0; P<0.0001).

For both patients with an NT-proBNP less than the median (difference, −4.1; 95% CI, −9.0 to 0.7; P=0.09) and patients with an NT-proBNP greater than the median (difference, −6.0; 95% CI, −12.0 to 0.1; P=0.053), treatment with LCZ696

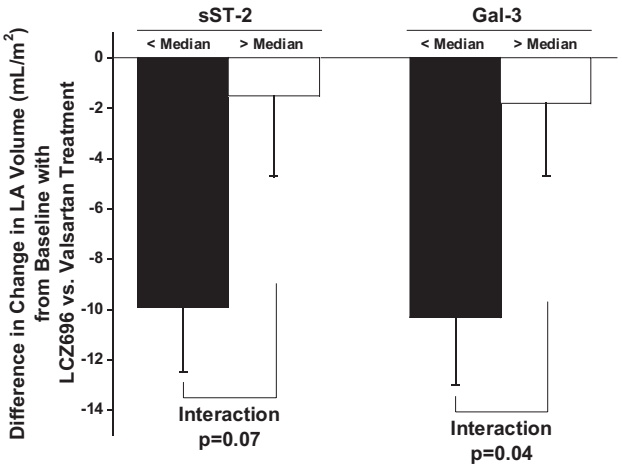

Figure 2. Change from baseline in left atrial volume (LAV) produced by treatment with LCZ696 vs valsartan in those with a baseline value of soluble form of ST2 (sST-2) and galectin 3 (Gal-3) above and below the median. When patients were divided into 2 groups, above and below the baseline median value of sST-2 or Gal-3, the effect on change in LA volume produced LCZ696 vs valsartan differed; in patients with a baseline value of sST-2 or Gal-3 above the median, treatment with LCZ696 did not result in a significant change in LA volume; in patients with a baseline value of sST-2 or Gal-3 below the median, treatment with LCZ696 resulted in a decrease in LA volume after 36 weeks of treatment compared with baseline.

resulted in a numerically greater reduction in LA volume from baseline compared with valsartan. A formal test of the interaction between randomized treatment and baseline NT-proBNP was not statistically significant (*P* for interaction=0.12). For patients with an eGFR less than the median (difference, −2.8; 95% CI, −7.7 to 2.1; *P*=0.26) and patients with an eGFR greater than the median (difference, −8.8; 95% CI, −15.0 to −2.6; *P*=0.006), treatment with LCZ696 resulted in a numerically greater reduction in LA volume from baseline compared with valsartan. This change was statistically significant for the group with an eGFR greater than the median. However, a formal test of interaction between randomized treatment and baseline eGFR was not statistically significant (*P* for interaction=0.58).

Interactions between LCZ696 and all other echocardiographic measurements of cardiac structure and function for the 4 biomarkers examined in this study were not performed because they were not listed as a priori end points and because our previous published studies showed that LCZ696 did not result in a change in any of these other echocardiographic parameters.[13]

There were no significant differences in any of the 4 biomarker values (sST-2, Gal-3, MMP-2, or PIIINP) between patients treated with valsartan versus LCZ696 at baseline or after 12 or 36 weeks of treatment (Table 3). This was true for the patient group as a whole and for subgroups with baseline sST-2 and Gal-3 above and below the observed median values. Comparing baseline biomarker values to after treatment values, MMP-2 increased significantly in both valsartan- and LCZ696-treated patients at week 36 versus baseline (*P*<0.001); there were no significant changes in the other 3 biomarkers comparing baseline with after treatment values.

History of atrial fibrillation was not associated with change in LA volume at 36 weeks *P*=0.12 and there was no interaction between randomized treatment and history of atrial fibrillation on change in LA volume *P*=0.44. In addition, for subjects with AF on ECG at baseline, the respective *P* values were also nonsignificant at 0.39 and 0.52.

Discussion

Data from this study support several novel and hypothesis generating findings. First, we found that patients with HFpEF had values of circulating biomarkers that may reflect a profibrotic state. Gal-3 and sST-2 were increased and MMP-2 was

Table 3. Serial Measurements of Plasma Biomarkers

	sST2	Galectin-3	MMP-2	PIIINP
Baseline				
Sample Size	296	294	247	178
All	33.0 (24.6–48.1)	17.8 (14.1–22.8)	188 (156–231)	5.6 (4.3–6.9)
Valsartan	33.8 (25.2–48.1)	16.9 (14.0–22.4)	188 (156–244)	5.6 (4.3–6.8)
LCZ696	32.2 (24.3–47.8)	18.9 (14.4–23.3)	187 (150–225)	5.5 (4.4–7.2)
12 wk				
Sample Size	262	250	244	
All	30.7 (23.4–44.7)	17.0 (13.9–22.1)	191 (155–234)	
Valsartan	31.0 (23.9–44.4)	17.1 (13.9–21.2)	194 (150–243)	
LCZ696	29.8 (23.3–45.8)	16.9 (14.2–22.2)	189 (158–222)	
36 wk				
Sample Size	211	214	241	182
All	33.4 (23.5–48.4)	16.8 (13.8–21.9)	253 (208–318)	5.3 (4.2–7.1)
Valsartan	35.2 (23.6–45.1)	16.8 (13.8–21.2)	261 (212–334)	5.4 (4.1–7.0)
LCZ696	31.4 (23.5–50.4)	17.0 (13.8–22.2)	248 (206–303)	5.3 (4.2–7.2)

All data are median (interquartile range) and in units of ng/mL. MMPs indicates matrix metaloproteinases; PIIINP, collagen III N-terminal propeptide; and sST-2, soluble form of ST2 (an interleukin-1 receptor family member).

decreased. Gal-3 and sST-2 have been shown to increase collagen synthesis in cardiac fibroblasts and MMP-2 has been shown to cause collagen degradation[2] (Figure 3). In aggregate, the directional changes in these biomarkers might be expected to be associated with an increase in myocardial collagen content. This study adds important, novel, clinically relevant data in a group of patients in which there is a large gap in knowledge. In particular, the panel of specific biomarkers used in this study has not been examined together in previous clinical studies or randomized clinic trials of patients with HFpEF. In addition, PARAMOUNT represents the only phase II randomized clinic trial of patients with HFpEF in which the prespecified primary end points have been positively improved by the therapy being tested. This provided a unique opportunity to examine the purposes proposed and test the hypotheses stated in this study.

The differences between biomarkers in the current HFpEF patients and referent controls are concordant with trends found in the limited number of other studies that included patients with HFpEF. For example, in studies including patients with HFpEF, sST-2 median values ranged from ≈25 to 30 ng/mL[2,29–31] and Gal-3 median values ranged from ≈12 to 14 ng/mL.[2,21,22,31] Variations in inclusion/exclusion criteria creating differences in population characteristics, comorbidity distribution, and severity of HF are likely responsible for small differences between studies.

For both sST-2 and Gal-3, there are Food and Drug Administration approved partition values that can be used in risk assessment analyses to predict morbid and mortal outcomes. When sST-2 is >35 ng/mL or Gal-3 is >17.8 ng/mL, there is an increase in risk. It should be noted; however, that both of these partition values were established from studies

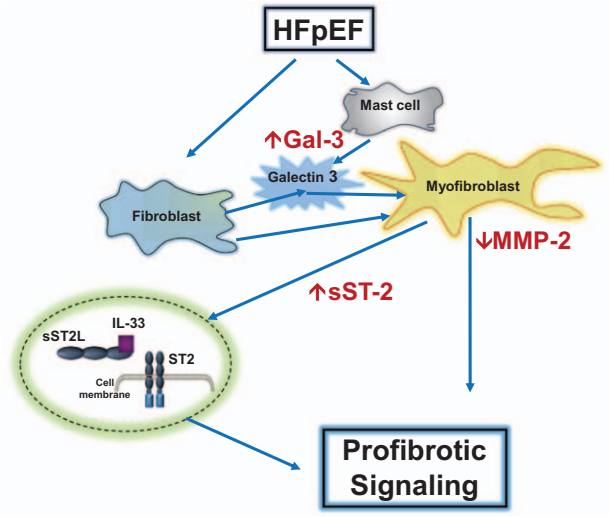

Figure 3. Schematic representation of mechanisms suggested by changes in circulating biomarkers. Increased galectin 3 (gal-3) secreted by mast cells may contribute to transdifferentiation of fibroblasts to myofibroblasts. Increased soluble form of ST2 (sST-2) may contribute to ST-2 profibrotic signaling. Decreased matrix metalloproteinase-2 (MMP-2) may contribute to less collagen degradation. In aggregate, these changes may contribute to increased myocardial extracellular matrix (ECM) collagen and fibrosis and may be reflected by the changes measured in circulating biomarkers. HFpEF indicates heart failure with preserved ejection fraction; and IL, interleukin.

such as HF-ACTION and others in which patients with HFrEF were exclusively or dominantly the focus of study. It is fortuitous that the median values for these 2 biomarkers in this study are identical to or close to the Food and Drug Administration approved partition values. Therefore, these data lend credence to the important biomarker observations made in this study of patients with HFpEF in PARAMOUNT.

Second, we found that biomarker levels correlated with indices of disease severity. The presence of more severe HFpEF is generally indicated by higher levels of NT-proBNP, diastolic function (such as E/E′ and LAV) and decreased renal function.[2,5,32–35] In this study, there was a direct relationship between sST-2 and each of these indices of disease severity; there was an inverse relationship with MMP-2. Thus, although each of the patients enrolled in PARAMOUNT had the clinical syndrome of HFpEF, those with the more severe disease had a biomarker pattern associated with a more profibrotic milieu. Although these interactions described in this study may have been expected, they have never been previously examined in a substantially sized study of patients with HFpEF. The interactions defined help to clarify signaling pathways that contribute to fibrosis-induced (and probably inflammation induced) abnormalities in structure and function in patients with HFpEF.

Third, the baseline pretreatment values of sST-2 and Gal-3 may have modified the response to LCZ696, specifically reduction in LA volume but not in NT-proBNP. Patients who had levels of sST-2 and Gal-3 below the observed median value showed a greater LA volume response to LCZ696 than those with sST-2 and Gal-3 above the median. These data are clearly not conclusive; however, they do allow the generation of important new hypotheses, particularly concerning the mechanisms underlying HFpEF and the effects of LCZ696 on these mechanisms. It is possible that patients with less severe myocardial fibrosis may be more responsive to treatment, particularly during a short period of treatment. Changes in LV structural remodeling may not be detectable until treatment has been continued for at least 12 months. Confirmation of these findings in a larger patient cohort and extension of treatment duration will clearly be needed in future studies to make more definitive conclusions. These kind of analyses are planned for Prospective Comparison of ARNI With ARB Global Outcomes in Heart Failure With Preserved Ejection Fraction (PARAGON-HF).

There are several possible factors that contribute to the fact that baseline biomarkers modified the response to LCZ696 on LAV but not to NT-proBNP. For example, changes in natriuretic peptides and changes in left atrial structure/function may reflect different but interdependent aspects of the pathophysiological mechanisms that underlie HFpEF. As presented in a previously published figure, LV diastolic filling pressures can be changed rapidly as a result of intravascular volume shifts and a change in operative compliance.[2] This hemodynamic change may be best reflected by changes in natriuretic peptides. LV diastolic filling pressures can also be changed more slowly by progressive fibrosis and a change in overall chamber compliance.[2] This structural change may be best reflected by changes in LAV. LCZ696 is likely to act on both of these mechanisms. Its direct diuretic, unloading, hemodynamic effect would be

expected to rapidly reduce filling volume, operative compliance, and NT-proBNP and to do so without needed to also affect structural changes in myocardial extracellular matrix collagen or LAV. Therefore, the extent of fibrosis (as reflected by sST-2 or Gal-3) should not modify the effects of LCZ696 on changes in NT-proBNP. By contrast, the effects of LCZ696 on the regression of fibrosis would be expected to occur over longer time periods. Therefore, patients with less fibrosis (as reflected by lower values of sST-2 or Gal-3) may respond more quickly with a decrease in overall chamber compliance and a resultant decrease in LAV because there was simply less fibrosis to regress. Patients with more fibrosis may take longer than 6 to 9 months to respond to LCZ696; however, this finding does not necessarily signal the absence of a response. There are few studies that document the time course of regression of fibrosis in pathophysiological processes that undergo treatments that effectively correct the pathophysiological abnormalities. However, perhaps the best example is the effects of aortic valve replacement in patients with aortic valve stenosis. In these studies, the complete reversal of LV fibrosis was time dependent and progressive during a 2- to 4-year period after aortic valve replacement.[36] Thus, the interplay between changes in hemodynamic and structural factors (and other factors) may contribute to the findings presented in this study.

Fourth, during the 36-week course of this study, sST-2, Gal-3, MMP-2, or PIIINP did not change in either the valsartan- or LCZ696-treated groups. The significance of this finding is not entirely clear. The same possible factors listed above may be applicable such as the length of the treatment period may be too short to see significant changes in these biomarkers. Conversely, it is possible that this particular set of biomarkers may have greater use as diagnostic, prognostic, and severity of illness indices rather than indices reflecting response to therapy.

Biomarkers Reflecting a Profibrotic State

Fibrillar collagen content can be altered by changes in the balance in the following processes: collagen synthesis, postsynthetic processing, and degradation. Biomarkers reflecting changes in these processes or their determinants were examined in PARAMOUNT.[2,5–7] For example, Gal-3, a β-galactoside–binding lectin, secreted by macrophages, may act to increase fibroblast proliferation, activity, transformation into myofibroblast, and increase collagen synthesis.[25–28,37–43] Likewise, soluble ST2, by acting as a decoy, prevents binding of IL-33 to membrane-bound ST2 and results in increased collagen synthesis (Figure 3). Therefore, both Gal-3 and sST2 induced increase in collagen synthesis would be expected to be reflected in an increase in levels of collagen propeptides, such as PIIINP (procollagen III N-terminal propeptide). However, in the presence of what seem to be profibrotic stimuli, we did not see changes in PIIINP. It is likely that the most important changes in myocardial collagen homeostasis involve a change in collagen I rather than collagen III, thus limiting the sensitivity of PIIINP versus measurements of collagen I propeptides. In addition to changes in synthesis, changes in degradation may affect collagen content. Insoluble collagen fibril degradation is caused by proteases such as MMPs, of which there are >29 known members.[5–11] Only MMP-2 was measured in PARAMOUNT; lower values seen in patients with HFpEF suggests decreased degradation rates; however, measurement of this single MMP particularly without measurement of tissue inhibitors of MMPs does not fully characterize the stoichiometric balance of this enzyme system.

Study Limitations

We readily acknowledge that comparing biomarker data from this study with nonsimultaneous, historic, previously published referent controls imposes clear limitations. Although a referent control group was not included in the design of the PARAMOUNT study, the previously published referent control subjects used for comparison were taken from subjects with an age, sex, and race distribution similar to this study population but with no evidence of active cardiovascular disease. In addition, the methodologies used in this study to measure biomarkers were either identical to or equivalent to the methods used in previously published studies. The small differences in methodologies are not likely to impose significant differences between study analyses.

Measurements of circulating biomarkers are not direct measurements of myocardial collagen homeostasis. Our analysis assumes that biomarkers are representatively excreted in a manner measureable in the circulation and that their predominant source is the myocardium. PARAMOUNT was designed with exclusion criteria that limited comorbid conditions that would produce nonmyocardial sources of circulating biomarkers that reflect changes in collagen homeostasis, such as severe renal, pulmonary, or hepatic fibrosis.

Conclusions

In patients with HFpEF, biomarkers that reflect collagen homeostasis correlated with the presence and severity of disease and underlying pathophysiology, and may modify the structural response to treatment.

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Disclosures

Drs Zile, Solomon, Pieske, Voors, and McMurray have received research support and have consulted for Novartis. Dr Jhund has consulted for Novartis. Drs Shi, Prescott, and Lefkowitz are employees of Novartis. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Heart failure with preserved ejection fraction (HFpEF) is a clinical syndrome that has been associated with changes in the extracellular matrix. The purpose of this analysis was to examine a selected portfolio of postulated profibrotic biomarkers in a defined population of HFpEF, relate these biomarkers to demographic characteristics, changes in left ventricular structure and function, severity of disease, and response to treatment. Data from this analysis support several novel and hypothesis-generating findings. First, patients with HFpEF have circulating biomarkers that reflect a profibrotic state. Galectin 3 and soluble form of ST2 were increased and matrix metalloproteinase-2 was decreased. In aggregate, these directional changes in these biomarkers might be expected to be associated with an increase in myocardial collagen content. Second, biomarker levels correlate with indices of disease severity. Although each of the patients enrolled in the Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction trial (PARAMOUNT) had the clinical syndrome of HFpEF, those with the more severe disease had a biomarker pattern associated with a more profibrotic milieu. Third, the baseline pretreatment values of soluble form of ST2 and galectin 3 may have modified the response to LCZ696, specifically reduction in left atrial volume. Patients who had levels of soluble form of ST2 and galectin 3 below the observed median value showed a greater left atrial volume response to LCZ696 than those with soluble form of ST2 and galectin 3 above the median. In patients with HFpEF, biomarkers that reflect collagen homeostasis correlated with the presence and severity of disease and underlying pathophysiology, and may modify the structural response to treatment.

Plasma Biomarkers Reflecting Profibrotic Processes in Heart Failure With a Preserved Ejection Fraction: Data From the Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction Study

Michael R. Zile, Pardeep S. Jhund, Catalin F. Baicu, Brian L. Claggett, Burkert Pieske, Adriaan A. Voors, Margaret F. Prescott, Victor Shi, Martin Lefkowitz, John J.V. McMurray and Scott D. Solomon
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SUPPLEMENTAL MATERIAL FOR

Plasma Biomarkers Reflecting Profibrotic Processes in Heart Failure with a Preserved Ejection Fraction: Data from the PARAMOUNT Study

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Supplemental TABLE 1. Baseline Characteristics According to GALECTIN-3 by Quartiles

	Q1, N=74	Q2, N=73	Q3, N=74	Q4, N=73	P for trend
Age, years	68 ± 10	73 ± 8	72 ± 9	72 ± 9	0.020
Women (%)	36 (48.6%)	39 (53.4%)	41 (55.4%)	49 (67.1%)	0.027
NYHA Class I	1 (1.4%)	1 (1.4%)	0 (0.0%)	0 (0.0%)	0.12
NYHA Class II	61 (82.4%)	58 (79.5%)	60 (81.1%)	54 (74.0%)	
NYHA Class III	12 (16.2%)	14 (19.2%)	14 (18.9%)	19 (26.0%)	
Previous admission for heart failure	24 (32.4%)	32 (43.8%)	26 (35.1%)	40 (54.8%)	0.023
History of atrial fibrillation	31 (41.9%)	29 (39.7%)	29 (39.2%)	33 (45.2%)	0.72
Atrial fibrillation at screening ECG	20 (27.0%)	20 (27.4%)	20 (27.0%)	23 (31.5%)	0.58
History of hypertension	66 (89.2%)	72 (98.6%)	66 (89.2%)	72 (98.6%)	0.13
History of diabetes	25 (33.8%)	24 (32.9%)	29 (39.2%)	33 (45.2%)	0.11
History of myocardial infarction	14 (18.9%)	17 (23.3%)	19 (25.7%)	9 (12.3%)	0.41
NT-proBNP (pg/mL), median [Q1, Q3]	693.0 [368.0, 1377.0]	867.0 [499.0, 1341.0]	909.5 [512.0, 1269.5]	962.0 [628.0, 1960.5]	0.014
Heart rate (bpm)	67.15 ± 11.76	68.03 ± 11.66	69.77 ± 12.18	72.26 ± 15.71	0.012
Body mass index (kg/m^2)	29.90 ± 5.60	29.60 ± 5.22	30.47 ± 5.87	30.00 ± 6.38	0.70
Systolic BP (mmHg)	134.65 ± 15.06	136.29 ± 14.17	136.10 ± 13.11	133.87 ± 13.66	0.73
Diastolic BP (mmHg)	79.31 ± 8.65	77.73 ± 9.79	77.88 ± 7.61	74.95 ± 10.93	0.008
eGFR (mL/min per 1.73 m²)	76.18 ± 20.31	68.40 ± 15.46	65.10 ± 17.35	51.13 ± 19.61	<0.001
ACE inhibitors or ARBs	67 (90.5%)	68 (93.2%)	73 (98.6%)	67 (91.8%)	0.47
Beta blockers	58 (78.4%)	61 (83.6%)	59 (79.7%)	53 (72.6%)	0.33
Aldosterone antagonists	9 (12.2%)	16 (21.9%)	19 (25.7%)	18 (24.7%)	0.05
E' (cm/s)	7.77 ± 2.92	7.02 ± 2.48	7.43 ± 3.05	7.64 ± 2.46	0.57
E/E'	11.41 ± 4.55	13.40 ± 6.89	12.74 ± 6.42	12.70 ± 5.69	0.17
LA volume index (mL/m²)	35.46 ± 12.54	35.90 ± 14.93	37.08 ± 15.57	34.50 ± 11.35	0.84
LV end diastolic volume (mL)	118.37 ± 29.41	115.44 ± 33.84	107.42 ± 26.09	107.70 ± 23.68	0.11
LV ejection fraction (%)	56.65 ± 7.00	57.89 ± 9.71	59.62 ± 7.16	58.81 ± 6.77	0.06
LV mass index (g/m²)	73.23 ± 17.35	81.46 ± 25.00	77.92 ± 21.16	77.39 ± 20.80	0.29
Relative wall thickness	0.35 ± 0.05	0.37 ± 0.07	0.37 ± 0.08	0.38 ± 0.08	0.004
Tricuspid regurgitant velocity (m/s)	2.43 ± 0.33	2.50 ± 0.36	2.60 ± 0.32	2.47 ± 0.49	0.31
LA strain (%)	22.10 ± 9.31	19.84 ± 7.15	22.34 ± 8.68	19.83 ± 6.24	0.40
LV global longitudinal strain (%)	-14.57 ± 3.52	-14.56 ± 3.20	-15.16 ± 3.48	-14.20 ± 3.09	0.79

Abbreviations:

ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular.

Supplemental TABLE 2. Baseline Characteristics According to MMP-2 Quartiles

	Q1 N=62	Q2 N=62	Q3 N=62	Q4 N=61	P for trend
Age, years	68 ± 10	72 ± 9	73 ± 8	73 ± 8	0.003
Women (%)	36 (58.1%)	32 (51.6%)	38 (61.3%)	34 (55.7%)	0.92
NYHA Class I	0 (0.0%)	1 (1.6%)	1 (1.6%)	0 (0.0%)	0.30
NYHA Class II	53 (85.5%)	51 (82.3%)	46 (74.2%)	49 (80.3%)	
NYHA Class III	9 (14.5%)	10 (16.1%)	15 (24.2%)	12 (19.7%)	
Previous admission for heart failure	26 (41.9%)	20 (32.3%)	24 (38.7%)	26 (42.6%)	0.76
History of atrial fibrillation	22 (35.5%)	30 (48.4%)	29 (46.8%)	27 (44.3%)	0.38
Atrial fibrillation at screening ECG	14 (22.6%)	22 (35.5%)	16 (25.8%)	21 (34.4%)	0.32
History of hypertension	58 (93.5%)	58 (93.5%)	57 (91.9%)	57 (93.4%)	0.89
History of diabetes	19 (30.6%)	21 (33.9%)	22 (35.5%)	27 (44.3%)	0.12
History of myocardial infarction	17 (27.4%)	16 (25.8%)	10 (16.1%)	9 (14.8%)	0.04
NT-proBNP (pg/mL), median [Q1, Q3]	573.0 [346.5, 1022.5]	766.5 [387.0, 1273.0]	973.5 [645.0, 1590.0]	1154.0 [744.0,1853.0]	<0.001
Heart rate (bpm)	69.87 ± 12.70	67.59 ± 11.40	68.53 ± 15.40	68.02 ± 11.90	0.53
Body mass index (kg/m^2)	30.34 ± 5.61	30.19 ± 5.24	31.17 ± 6.15	30.11 ± 5.50	0.92
Systolic BP (mmHg)	135.13 ± 14.22	136.55 ± 14.61	133.15 ± 12.00	137.16 ± 15.22	0.74
Diastolic BP (mmHg)	80.27 ± 9.17	78.62 ± 10.01	76.57 ± 8.45	74.00 ± 10.05	<0.001
eGFR (mL/min per 1.73 m²)	71.82 ± 21.46	66.18 ± 17.27	65.97 ± 22.51	59.52 ± 16.78	0.001
ACE inhibitors or ARBs	60 (96.8%)	56 (90.3%)	57 (91.9%)	57 (93.4%)	0.56
Beta blockers	47 (75.8%)	50 (80.6%)	50 (80.6%)	54 (88.5%)	0.09
Aldosterone antagonists	14 (22.6%)	11 (17.7%)	16 (25.8%)	13 (21.3%)	0.86
E' (cm/s)	6.85 ± 2.74	7.44 ± 2.91	6.92 ± 2.59	8.45 ± 2.80	0.17
E/E'	11.44 ± 5.18	11.72 ± 5.38	14.86 ± 6.77	12.79 ± 6.35	0.013
LA volume index (mL/m²)	33.13 ± 13.09	35.78 ± 13.58	37.12 ± 14.13	38.03 ± 13.32	0.042
LV end diastolic volume (mL)	114.89 ± 31.94	115.91 ± 26.66	106.07 ± 24.37	114.51 ± 31.76	0.31
LV ejection fraction (%)	57.47 ± 8.54	57.86 ± 8.95	59.19 ± 7.48	59.87 ± 6.08	0.028
LV mass index (g/m²)	75.58 ± 20.12	78.31 ± 21.32	75.04 ± 20.53	79.58 ± 21.49	0.98
Relative wall thickness	0.37 ± 0.06	0.37 ± 0.07	0.37 ± 0.05	0.38 ± 0.09	0.48
Tricuspid regurgitant velocity (m/s)	2.41 ± 0.29	2.35 ± 0.15	2.58 ± 0.45	2.64 ± 0.45	0.004
LA strain (%)	22.57 ± 7.70	21.24 ± 8.72	20.72 ± 6.92	20.55 ± 8.61	0.22
LV global longitudinal strain (%)	-15.25 ± 3.85	-15.03 ± 2.97	-14.42 ± 3.18	-15.18 ± 3.09	0.68

Abbreviations:

ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular.

Supplemental TABLE 3. Baseline Characteristics According to PIIINP Quartiles

	Q1 N=45	Q2 N=44	Q3 N=44	Q4 N=45	P for trend
Age, years	70 ± 10	70 ± 11	72 ± 8	73 ± 9	0.06
Women (%)	22 (48.9%)	25 (56.8%)	25 (55.6%)	27 (61.4%)	0.28
NYHA Class I	0 (0.0%)	0 (0.0%)	1 (2.2%)	1 (2.3%)	0.88
NYHA Class II	37 (82.2%)	34 (77.3%)	36 (80.0%)	34 (77.3%)	
NYHA Class III	8 (17.8%)	10 (22.7%)	8 (17.8%)	9 (20.5%)	
Previous admission for heart failure	18 (40.0%)	14 (31.8%)	20 (44.4%)	9 (20.5%)	0.15
History of atrial fibrillation	20 (44.4%)	19 (43.2%)	23 (51.1%)	20 (45.5%)	0.74
Atrial fibrillation at screening ECG	15 (33.3%)	13 (29.5%)	17 (37.8%)	12 (27.3%)	0.76
History of hypertension	44 (97.8%)	40 (90.9%)	40 (88.9%)	43 (97.7%)	0.88
History of diabetes	23 (51.1%)	10 (22.7%)	14 (31.1%)	14 (31.8%)	0.12
History of myocardial infarction	15 (33.3%)	13 (29.5%)	7 (15.6%)	8 (18.2%)	0.142
NT-proBNP (pg/mL), median [Q1, Q3]	511.0 [369.0, 1018.0]	796.0 [517.0, 1267.0]	1107.0 [572.0,1800.0]	967.0 [625.0, 1507.0]	0.001
Heart rate (bpm)	67.73 ± 12.54	65.09 ± 9.77	68.11 ± 11.91	73.36 ± 15.13	0.020
Body mass index (kg/m^2)	30.40 ± 4.38	30.32 ± 5.18	29.90 ± 5.84	31.79 ± 6.67	0.32
Systolic BP (mmHg)	135.47 ± 12.81	133.42 ± 13.05	137.43 ± 13.96	133.87 ± 11.51	0.93
Diastolic BP (mmHg)	79.79 ± 8.76	77.58 ± 9.56	78.32 ± 10.12	76.88 ± 9.76	0.21
eGFR (mL/min per 1.73 m²)	72.20 ± 18.41	70.14 ± 20.06	65.82 ± 18.07	63.79 ± 17.54	0.019
ACE inhibitors or ARBs	40 (88.9%)	43 (97.7%)	43 (95.6%)	41 (93.2%)	0.50
Beta blockers	37 (82.2%)	34 (77.3%)	40 (88.9%)	35 (79.5%)	0.89
Aldosterone antagonists	9 (20.0%)	11 (25.0%)	12 (26.7%)	6 (13.6%)	0.54
E' (cm/s)	7.33 ± 2.70	7.58 ± 2.73	7.57 ± 2.98	8.19 ± 2.90	0.30
E/E'	11.74 ± 5.66	13.39 ± 7.19	13.06 ± 5.72	11.03 ± 4.85	0.56
LA volume index (mL/m²)	32.74 ± 12.62	38.87 ± 13.36	37.76 ± 13.05	33.83 ± 16.06	0.83
LV end diastolic volume (mL)	122.27 ± 31.17	108.15 ± 27.74	116.64 ± 31.47	108.56 ± 22.50	0.16
LV ejection fraction (%)	57.16 ± 8.41	57.87 ± 6.81	58.82 ± 9.70	60.45 ± 7.71	0.033
LV mass index (g/m²)	74.45 ± 23.32	75.40 ± 17.25	73.08 ± 20.08	75.05 ± 20.04	0.96
Relative wall thickness	0.35 ± 0.08	0.35 ± 0.06	0.37 ± 0.06	0.37 ± 0.06	0.17
Tricuspid regurgitant velocity (m/s)	2.42 ± 0.35	2.53 ± 0.36	2.51 ± 0.36	2.52 ± 0.30	0.68
LA strain (%)	22.78 ± 7.44	18.80 ± 7.71	21.96 ± 8.89	23.31 ± 8.26	0.52
LV global longitudinal strain (%)	-15.39 ± 3.17	-14.67 ± 2.96	-15.22 ± 3.22	-15.55 ± 2.77	0.72

Abbreviations:

ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular.

Supplemental TABLE 4. Baseline Characteristics According to sST-2 Quartiles

	Q1 N=74	Q2 N=74	Q3 N=74	Q4 N=74	P for trend
Age, years	69 ± 10	70 ± 10	71 ± 8	74 ± 8	0.004
Women (%)	47 (63.5%)	45 (60.8%)	38 (51.4%)	36 (48.6%)	0.036
NYHA Class I	0 (0.0%)	1 (1.4%)	1 (1.4%)	0 (0.0%)	0.043
NYHA Class II	66 (89.2%)	55 (74.3%)	59 (79.7%)	54 (73.0%)	
NYHA Class III	8 (10.8%)	18 (24.3%)	14 (18.9%)	20 (27.0%)	
Previous admission for heart failure	28 (37.8%)	29 (39.2%)	33 (44.6%)	34 (45.9%)	0.25
History of atrial fibrillation	17 (23.0%)	32 (43.2%)	33 (44.6%)	39 (52.7%)	<0.001
Atrial fibrillation at screening ECG	7 (9.5%)	20 (27.0%)	22 (29.7%)	33 (44.6%)	<0.001
History of hypertension	66 (89.2%)	72 (97.3%)	73 (98.6%)	67 (90.5%)	0.66
History of diabetes	26 (35.1%)	21 (28.4%)	34 (45.9%)	32 (43.2%)	0.10
History of myocardial infarction	20 (27.0%)	14 (18.9%)	13 (17.6%)	14 (18.9%)	0.22
NT-proBNP (pg/mL), median [Q1, Q3]	773.5 [355.0, 1247.5]	715.5 [393.0, 1341.0]	957.0 [601.0, 1322.0]	1014.0 [603.0,1800.0]	0.002
Heart rate (bpm)	69.23 ± 13.28	67.59 ± 10.67	70.18 ± 14.45	70.01 ± 13.46	0.47
Body mass index (kg/m^2)	28.57 ± 5.26	30.52 ± 6.23	30.61 ± 5.38	30.24 ± 6.09	0.09
Systolic BP (mmHg)	134.34 ± 13.40	137.82 ± 13.19	134.34 ± 13.62	134.84 ± 15.93	0.79
Diastolic BP (mmHg)	77.78 ± 7.73	80.02 ± 10.24	77.79 ± 8.03	73.79 ± 10.60	0.004
eGFR (mL/min per 1.73 m²)	69.12 ± 21.46	68.47 ± 19.88	62.32 ± 20.74	61.19 ± 18.14	0.005
ACE inhibitors or ARBs	72 (97.3%)	69 (93.2%)	67 (90.5%)	68 (91.9%)	0.15
Beta blockers	55 (74.3%)	58 (78.4%)	61 (82.4%)	59 (79.7%)	0.34
Aldosterone antagonists	9 (12.2%)	13 (17.6%)	22 (29.7%)	18 (24.3%)	0.021
E' (cm/s)	7.10 ± 2.69	7.43 ± 2.77	7.72 ± 2.60	7.54 ± 2.99	0.30
E/E'	10.98 ± 3.87	12.57 ± 6.00	13.29 ± 6.85	13.70 ± 6.63	0.06
LA volume index (mL/m²)	31.67 ± 11.70	35.10 ± 12.93	36.40 ± 13.94	39.83 ± 14.90	<0.001
LV end diastolic volume (mL)	114.79 ± 29.12	106.59 ± 22.81	111.27 ± 29.73	120.46 ± 33.43	0.35
LV ejection fraction (%)	57.88 ± 9.10	58.83 ± 7.20	58.46 ± 7.25	57.33 ± 7.59	0.71
LV mass index (g/m²)	76.34 ± 18.92	77.54 ± 21.03	79.36 ± 25.66	77.16 ± 18.49	0.76
Relative wall thickness	0.37 ± 0.07	0.36 ± 0.06	0.37 ± 0.08	0.37 ± 0.06	0.61
Tricuspid regurgitant velocity (m/s)	2.38 ± 0.29	2.54 ± 0.31	2.52 ± 0.40	2.58 ± 0.44	0.42
LA strain (%)	23.94 ± 8.92	20.15 ± 7.80	21.04 ± 7.53	19.78 ± 7.46	0.025
LV global longitudinal strain (%)	-14.56 ± 3.61	-14.19 ± 3.02	-15.06 ± 3.51	-14.85 ± 3.21	0.39

Abbreviations:

sST-2 = soluble form of ST2(an Interleukin-1 receptor family member), ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular

SUPPLEMENTAL MATERIAL FOR

Plasma Biomarkers Reflecting Profibrotic Processes in Heart Failure with a Preserved Ejection Fraction: Data from the PARAMOUNT Study

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Supplemental TABLE 1. Baseline Characteristics According to GALECTIN-3 by Quartiles

	Q1, N=74	Q2, N=73	Q3, N=74	Q4, N=73	P for trend
Age, years	68 ± 10	73 ± 8	72 ± 9	72 ± 9	0.020
Women (%)	36 (48.6%)	39 (53.4%)	41 (55.4%)	49 (67.1%)	0.027
NYHA Class I	1 (1.4%)	1 (1.4%)	0 (0.0%)	0 (0.0%)	0.12
NYHA Class II	61 (82.4%)	58 (79.5%)	60 (81.1%)	54 (74.0%)	
NYHA Class III	12 (16.2%)	14 (19.2%)	14 (18.9%)	19 (26.0%)	
Previous admission for heart failure	24 (32.4%)	32 (43.8%)	26 (35.1%)	40 (54.8%)	0.023
History of atrial fibrillation	31 (41.9%)	29 (39.7%)	29 (39.2%)	33 (45.2%)	0.72
Atrial fibrillation at screening ECG	20 (27.0%)	20 (27.4%)	20 (27.0%)	23 (31.5%)	0.58
History of hypertension	66 (89.2%)	72 (98.6%)	66 (89.2%)	72 (98.6%)	0.13
History of diabetes	25 (33.8%)	24 (32.9%)	29 (39.2%)	33 (45.2%)	0.11
History of myocardial infarction	14 (18.9%)	17 (23.3%)	19 (25.7%)	9 (12.3%)	0.41
NT-proBNP (pg/mL), median [Q1, Q3]	693.0 [368.0, 1377.0]	867.0 [499.0, 1341.0]	909.5 [512.0, 1269.5]	962.0 [628.0, 1960.5]	0.014
Heart rate (bpm)	67.15 ± 11.76	68.03 ± 11.66	69.77 ± 12.18	72.26 ± 15.71	0.012
Body mass index (kg/m^2)	29.90 ± 5.60	29.60 ± 5.22	30.47 ± 5.87	30.00 ± 6.38	0.70
Systolic BP (mmHg)	134.65 ± 15.06	136.29 ± 14.17	136.10 ± 13.11	133.87 ± 13.66	0.73
Diastolic BP (mmHg)	79.31 ± 8.65	77.73 ± 9.79	77.88 ± 7.61	74.95 ± 10.93	0.008
eGFR (mL/min per 1.73 m²)	76.18 ± 20.31	68.40 ± 15.46	65.10 ± 17.35	51.13 ± 19.61	<0.001
ACE inhibitors or ARBs	67 (90.5%)	68 (93.2%)	73 (98.6%)	67 (91.8%)	0.47
Beta blockers	58 (78.4%)	61 (83.6%)	59 (79.7%)	53 (72.6%)	0.33
Aldosterone antagonists	9 (12.2%)	16 (21.9%)	19 (25.7%)	18 (24.7%)	0.05
E' (cm/s)	7.77 ± 2.92	7.02 ± 2.48	7.43 ± 3.05	7.64 ± 2.46	0.57
E/E'	11.41 ± 4.55	13.40 ± 6.89	12.74 ± 6.42	12.70 ± 5.69	0.17
LA volume index (mL/m²)	35.46 ± 12.54	35.90 ± 14.93	37.08 ± 15.57	34.50 ± 11.35	0.84
LV end diastolic volume (mL)	118.37 ± 29.41	115.44 ± 33.84	107.42 ± 26.09	107.70 ± 23.68	0.11
LV ejection fraction (%)	56.65 ± 7.00	57.89 ± 9.71	59.62 ± 7.16	58.81 ± 6.77	0.06
LV mass index (g/m²)	73.23 ± 17.35	81.46 ± 25.00	77.92 ± 21.16	77.39 ± 20.80	0.29
Relative wall thickness	0.35 ± 0.05	0.37 ± 0.07	0.37 ± 0.08	0.38 ± 0.08	0.004
Tricuspid regurgitant velocity (m/s)	2.43 ± 0.33	2.50 ± 0.36	2.60 ± 0.32	2.47 ± 0.49	0.31
LA strain (%)	22.10 ± 9.31	19.84 ± 7.15	22.34 ± 8.68	19.83 ± 6.24	0.40
LV global longitudinal strain (%)	-14.57 ± 3.52	-14.56 ± 3.20	-15.16 ± 3.48	-14.20 ± 3.09	0.79

Abbreviations:

ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular.

Supplemental TABLE 2. Baseline Characteristics According to MMP-2 Quartiles

	Q1 N=62	Q2 N=62	Q3 N=62	Q4 N=61	P for trend
Age, years	68 ± 10	72 ± 9	73 ± 8	73 ± 8	0.003
Women (%)	36 (58.1%)	32 (51.6%)	38 (61.3%)	34 (55.7%)	0.92
NYHA Class I	0 (0.0%)	1 (1.6%)	1 (1.6%)	0 (0.0%)	0.30
NYHA Class II	53 (85.5%)	51 (82.3%)	46 (74.2%)	49 (80.3%)	
NYHA Class III	9 (14.5%)	10 (16.1%)	15 (24.2%)	12 (19.7%)	
Previous admission for heart failure	26 (41.9%)	20 (32.3%)	24 (38.7%)	26 (42.6%)	0.76
History of atrial fibrillation	22 (35.5%)	30 (48.4%)	29 (46.8%)	27 (44.3%)	0.38
Atrial fibrillation at screening ECG	14 (22.6%)	22 (35.5%)	16 (25.8%)	21 (34.4%)	0.32
History of hypertension	58 (93.5%)	58 (93.5%)	57 (91.9%)	57 (93.4%)	0.89
History of diabetes	19 (30.6%)	21 (33.9%)	22 (35.5%)	27 (44.3%)	0.12
History of myocardial infarction	17 (27.4%)	16 (25.8%)	10 (16.1%)	9 (14.8%)	0.04
NT-proBNP (pg/mL), median [Q1, Q3]	573.0 [346.5, 1022.5]	766.5 [387.0, 1273.0]	973.5 [645.0, 1590.0]	1154.0 [744.0,1853.0]	<0.001
Heart rate (bpm)	69.87 ± 12.70	67.59 ± 11.40	68.53 ± 15.40	68.02 ± 11.90	0.53
Body mass index (kg/m^2)	30.34 ± 5.61	30.19 ± 5.24	31.17 ± 6.15	30.11 ± 5.50	0.92
Systolic BP (mmHg)	135.13 ± 14.22	136.55 ± 14.61	133.15 ± 12.00	137.16 ± 15.22	0.74
Diastolic BP (mmHg)	80.27 ± 9.17	78.62 ± 10.01	76.57 ± 8.45	74.00 ± 10.05	<0.001
eGFR (mL/min per 1.73 m²)	71.82 ± 21.46	66.18 ± 17.27	65.97 ± 22.51	59.52 ± 16.78	0.001
ACE inhibitors or ARBs	60 (96.8%)	56 (90.3%)	57 (91.9%)	57 (93.4%)	0.56
Beta blockers	47 (75.8%)	50 (80.6%)	50 (80.6%)	54 (88.5%)	0.09
Aldosterone antagonists	14 (22.6%)	11 (17.7%)	16 (25.8%)	13 (21.3%)	0.86
E' (cm/s)	6.85 ± 2.74	7.44 ± 2.91	6.92 ± 2.59	8.45 ± 2.80	0.17
E/E'	11.44 ± 5.18	11.72 ± 5.38	14.86 ± 6.77	12.79 ± 6.35	0.013
LA volume index (mL/m²)	33.13 ± 13.09	35.78 ± 13.58	37.12 ± 14.13	38.03 ± 13.32	0.042
LV end diastolic volume (mL)	114.89 ± 31.94	115.91 ± 26.66	106.07 ± 24.37	114.51 ± 31.76	0.31
LV ejection fraction (%)	57.47 ± 8.54	57.86 ± 8.95	59.19 ± 7.48	59.87 ± 6.08	0.028
LV mass index (g/m²)	75.58 ± 20.12	78.31 ± 21.32	75.04 ± 20.53	79.58 ± 21.49	0.98
Relative wall thickness	0.37 ± 0.06	0.37 ± 0.07	0.37 ± 0.05	0.38 ± 0.09	0.48
Tricuspid regurgitant velocity (m/s)	2.41 ± 0.29	2.35 ± 0.15	2.58 ± 0.45	2.64 ± 0.45	0.004
LA strain (%)	22.57 ± 7.70	21.24 ± 8.72	20.72 ± 6.92	20.55 ± 8.61	0.22
LV global longitudinal strain (%)	-15.25 ± 3.85	-15.03 ± 2.97	-14.42 ± 3.18	-15.18 ± 3.09	0.68

Abbreviations:

ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular.

Supplemental TABLE 3. Baseline Characteristics According to PIIINP Quartiles

	Q1 N=45	Q2 N=44	Q3 N=44	Q4 N=45	P for trend
Age, years	70 ± 10	70 ± 11	72 ± 8	73 ± 9	0.06
Women (%)	22 (48.9%)	25 (56.8%)	25 (55.6%)	27 (61.4%)	0.28
NYHA Class I	0 (0.0%)	0 (0.0%)	1 (2.2%)	1 (2.3%)	0.88
NYHA Class II	37 (82.2%)	34 (77.3%)	36 (80.0%)	34 (77.3%)	
NYHA Class III	8 (17.8%)	10 (22.7%)	8 (17.8%)	9 (20.5%)	
Previous admission for heart failure	18 (40.0%)	14 (31.8%)	20 (44.4%)	9 (20.5%)	0.15
History of atrial fibrillation	20 (44.4%)	19 (43.2%)	23 (51.1%)	20 (45.5%)	0.74
Atrial fibrillation at screening ECG	15 (33.3%)	13 (29.5%)	17 (37.8%)	12 (27.3%)	0.76
History of hypertension	44 (97.8%)	40 (90.9%)	40 (88.9%)	43 (97.7%)	0.88
History of diabetes	23 (51.1%)	10 (22.7%)	14 (31.1%)	14 (31.8%)	0.12
History of myocardial infarction	15 (33.3%)	13 (29.5%)	7 (15.6%)	8 (18.2%)	0.142
NT-proBNP (pg/mL), median [Q1, Q3]	511.0 [369.0, 1018.0]	796.0 [517.0, 1267.0]	1107.0 [572.0,1800.0]	967.0 [625.0, 1507.0]	0.001
Heart rate (bpm)	67.73 ± 12.54	65.09 ± 9.77	68.11 ± 11.91	73.36 ± 15.13	0.020
Body mass index (kg/m^2)	30.40 ± 4.38	30.32 ± 5.18	29.90 ± 5.84	31.79 ± 6.67	0.32
Systolic BP (mmHg)	135.47 ± 12.81	133.42 ± 13.05	137.43 ± 13.96	133.87 ± 11.51	0.93
Diastolic BP (mmHg)	79.79 ± 8.76	77.58 ± 9.56	78.32 ± 10.12	76.88 ± 9.76	0.21
eGFR (mL/min per 1.73 m²)	72.20 ± 18.41	70.14 ± 20.06	65.82 ± 18.07	63.79 ± 17.54	0.019
ACE inhibitors or ARBs	40 (88.9%)	43 (97.7%)	43 (95.6%)	41 (93.2%)	0.50
Beta blockers	37 (82.2%)	34 (77.3%)	40 (88.9%)	35 (79.5%)	0.89
Aldosterone antagonists	9 (20.0%)	11 (25.0%)	12 (26.7%)	6 (13.6%)	0.54
E' (cm/s)	7.33 ± 2.70	7.58 ± 2.73	7.57 ± 2.98	8.19 ± 2.90	0.30
E/E'	11.74 ± 5.66	13.39 ± 7.19	13.06 ± 5.72	11.03 ± 4.85	0.56
LA volume index (mL/m²)	32.74 ± 12.62	38.87 ± 13.36	37.76 ± 13.05	33.83 ± 16.06	0.83
LV end diastolic volume (mL)	122.27 ± 31.17	108.15 ± 27.74	116.64 ± 31.47	108.56 ± 22.50	0.16
LV ejection fraction (%)	57.16 ± 8.41	57.87 ± 6.81	58.82 ± 9.70	60.45 ± 7.71	0.033
LV mass index (g/m²)	74.45 ± 23.32	75.40 ± 17.25	73.08 ± 20.08	75.05 ± 20.04	0.96
Relative wall thickness	0.35 ± 0.08	0.35 ± 0.06	0.37 ± 0.06	0.37 ± 0.06	0.17
Tricuspid regurgitant velocity (m/s)	2.42 ± 0.35	2.53 ± 0.36	2.51 ± 0.36	2.52 ± 0.30	0.68
LA strain (%)	22.78 ± 7.44	18.80 ± 7.71	21.96 ± 8.89	23.31 ± 8.26	0.52
LV global longitudinal strain (%)	-15.39 ± 3.17	-14.67 ± 2.96	-15.22 ± 3.22	-15.55 ± 2.77	0.72

Abbreviations:

ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular.

Supplemental TABLE 4. Baseline Characteristics According to sST-2 Quartiles

	Q1 N=74	Q2 N=74	Q3 N=74	Q4 N=74	P for trend
Age, years	69 ± 10	70 ± 10	71 ± 8	74 ± 8	0.004
Women (%)	47 (63.5%)	45 (60.8%)	38 (51.4%)	36 (48.6%)	0.036
NYHA Class I	0 (0.0%)	1 (1.4%)	1 (1.4%)	0 (0.0%)	0.043
NYHA Class II	66 (89.2%)	55 (74.3%)	59 (79.7%)	54 (73.0%)	
NYHA Class III	8 (10.8%)	18 (24.3%)	14 (18.9%)	20 (27.0%)	
Previous admission for heart failure	28 (37.8%)	29 (39.2%)	33 (44.6%)	34 (45.9%)	0.25
History of atrial fibrillation	17 (23.0%)	32 (43.2%)	33 (44.6%)	39 (52.7%)	<0.001
Atrial fibrillation at screening ECG	7 (9.5%)	20 (27.0%)	22 (29.7%)	33 (44.6%)	<0.001
History of hypertension	66 (89.2%)	72 (97.3%)	73 (98.6%)	67 (90.5%)	0.66
History of diabetes	26 (35.1%)	21 (28.4%)	34 (45.9%)	32 (43.2%)	0.10
History of myocardial infarction	20 (27.0%)	14 (18.9%)	13 (17.6%)	14 (18.9%)	0.22
NT-proBNP (pg/mL), median [Q1, Q3]	773.5 [355.0, 1247.5]	715.5 [393.0, 1341.0]	957.0 [601.0, 1322.0]	1014.0 [603.0,1800.0]	0.002
Heart rate (bpm)	69.23 ± 13.28	67.59 ± 10.67	70.18 ± 14.45	70.01 ± 13.46	0.47
Body mass index (kg/m^2)	28.57 ± 5.26	30.52 ± 6.23	30.61 ± 5.38	30.24 ± 6.09	0.09
Systolic BP (mmHg)	134.34 ± 13.40	137.82 ± 13.19	134.34 ± 13.62	134.84 ± 15.93	0.79
Diastolic BP (mmHg)	77.78 ± 7.73	80.02 ± 10.24	77.79 ± 8.03	73.79 ± 10.60	0.004
eGFR (mL/min per 1.73 m²)	69.12 ± 21.46	68.47 ± 19.88	62.32 ± 20.74	61.19 ± 18.14	0.005
ACE inhibitors or ARBs	72 (97.3%)	69 (93.2%)	67 (90.5%)	68 (91.9%)	0.15
Beta blockers	55 (74.3%)	58 (78.4%)	61 (82.4%)	59 (79.7%)	0.34
Aldosterone antagonists	9 (12.2%)	13 (17.6%)	22 (29.7%)	18 (24.3%)	0.021
E' (cm/s)	7.10 ± 2.69	7.43 ± 2.77	7.72 ± 2.60	7.54 ± 2.99	0.30
E/E'	10.98 ± 3.87	12.57 ± 6.00	13.29 ± 6.85	13.70 ± 6.63	0.06
LA volume index (mL/m²)	31.67 ± 11.70	35.10 ± 12.93	36.40 ± 13.94	39.83 ± 14.90	<0.001
LV end diastolic volume (mL)	114.79 ± 29.12	106.59 ± 22.81	111.27 ± 29.73	120.46 ± 33.43	0.35
LV ejection fraction (%)	57.88 ± 9.10	58.83 ± 7.20	58.46 ± 7.25	57.33 ± 7.59	0.71
LV mass index (g/m²)	76.34 ± 18.92	77.54 ± 21.03	79.36 ± 25.66	77.16 ± 18.49	0.76
Relative wall thickness	0.37 ± 0.07	0.36 ± 0.06	0.37 ± 0.08	0.37 ± 0.06	0.61
Tricuspid regurgitant velocity (m/s)	2.38 ± 0.29	2.54 ± 0.31	2.52 ± 0.40	2.58 ± 0.44	0.42
LA strain (%)	23.94 ± 8.92	20.15 ± 7.80	21.04 ± 7.53	19.78 ± 7.46	0.025
LV global longitudinal strain (%)	-14.56 ± 3.61	-14.19 ± 3.02	-15.06 ± 3.51	-14.85 ± 3.21	0.39

Abbreviations:

sST-2 = soluble form of ST2(an Interleukin-1 receptor family member), ACE = Angiotensin converting enzyme ARB = angiotensin receptor blocker, eGFR = estimated glomerular filtration rate, NYHA = New York Heart Association, NT-proBNP = N-terminal pro-B-type natriuretic peptide, BP = blood pressure, continuous data are presented as mean ± standard deviation, categorical data are presented as frequency and % of total. E = early diastolic filling velocity, E' = early diastolic myocardial velocity, LA = left atrium, LV = left ventricular