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Effect of Sacubitril/Valsartan on Biomarkers of Extracellular Matrix Regulation in Patients With HFpEF

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ABSTRACT

BACKGROUND Myocardial fibrosis may contribute to the pathophysiology of heart failure with preserved ejection fraction. Given the biochemical targets of sacubitril/valsartan, this study hypothesized that circulating biomarkers reflecting the mechanisms that determine extracellular matrix homeostasis are altered by sacubitril/valsartan compared with valsartan alone.

OBJECTIVES This study investigated the effects of sacubitril/valsartan on biomarkers of extracellular matrix homeostasis and the association between biomarkers and the primary endpoint (total heart failure hospitalizations and cardiovascular death).

METHODS N-terminal propeptide of collagen I and III, tissue inhibitor of matrix metalloproteinase 1, carboxyl-terminal telopeptide of collagen type I, and soluble ST2 were measured at baseline (n = 1,135) and 16 (n = 1,113) and 48 weeks (n = 1,016) after randomization. The effects of sacubitril/valsartan on these biomarkers were compared with those of valsartan alone. Baseline biomarker values and changes from baseline to 16 weeks were related to primary endpoint.

RESULTS At baseline, all 5 biomarkers were higher than published referent control values. Sixteen weeks after randomization, sacubitril/valsartan decreased tissue inhibitor of matrix metalloproteinase 1 by 8% (95% confidence interval [CI]: 6% to 10%; $p < 0.001$), soluble ST2 by 4% (95% CI: 1% to 7%; $p = 0.002$), and N-terminal propeptide of collagen III by 3% (95% CI: 0% to 6%; $p = 0.04$) and increased carboxyl-terminal telopeptide of collagen type I by 4% (95% CI: 1% to 8%; $p = 0.02$) compared with valsartan alone, consistently in men and women and patients with left ventricular ejection fraction above or below the median of 57%. Higher levels of tissue inhibitor of matrix metalloproteinase 1 and soluble ST2 at baseline and increases in these markers at 16 weeks were associated with higher primary endpoint event rates.

CONCLUSIONS Biomarkers reflecting extracellular matrix homeostasis are elevated in heart failure with preserved ejection fraction, favorably altered by sacubitril/valsartan, and have important prognostic value. (Prospective Comparison of ARNI With ARB Global Outcomes in HF With Preserved Ejection Fraction [PARAGON-HF]; NCT01920711) (J Am Coll Cardiol 2020;76:503–14) © 2020 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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ABBREVIATIONS AND ACRONYMS

CI = confidence interval

CITP = collagen I telopeptide

CV = cardiovascular

ECM = extracellular matrix

HF = heart failure

HFpEF = heart failure with a preserved ejection fraction

HFrEF = heart failure with reduced ejection fraction

hsTNT = high-sensitivity troponin T

LVEF = left ventricular ejection fraction

MMP = matrix metalloproteinase

NT-proBNP = N-terminal pro-B-type natriuretic peptide

PIIINP = N-terminal propeptide of collagen III

PINP = N-terminal propeptide of collagen I

RR = rate ratio

sST2 = soluble ST2

TIMP = tissue inhibitor of matrix metalloproteinase

Changes in the architecture, composition, and distribution of the myocardial extracellular matrix (ECM) play a central role in myocardial remodeling and left ventricular diastolic dysfunction (1–3). A change in ECM homeostatic control is postulated to occur during the development and progression of heart failure (HF) in patients with heart failure with reduced ejection fraction (HFrEF) and in those with heart failure with preserved ejection fraction (HFpEF). The changes in ECM homeostasis that lead to structural remodeling include changes in synthesis, processing, degradation, and turnover of proteins such as fibrillar collagen (4).

SEE PAGE 515

Although these changes occur within the myocardium itself, evidence of their occurrence can also be detected by examining circulating biomarkers (5–9). For example, maturation of newly synthesized collagen requires removal of the N-terminal and C-terminal propeptides; the concentration of these propeptides (such as N-terminal propeptide of collagen I [PINP] and N-terminal propeptide of collagen III [PIIINP]) reflect collagen synthesis rate. Activation of the principal collagen-producing myocardial cell, the fibroblast, is enhanced by soluble ST2 (sST2). Fibrillar collagen turnover and degradation result in liberation of collagen I telopeptides (CITPs). Collagen degradation by matrix metalloproteinases (MMPs) is inhibited by tissue inhibitor of matrix metalloproteinase (TIMP)-1.

Sacubitril/valsartan increases levels of vasoactive peptides including natriuretic peptides that may inhibit fibrosis (10). We therefore hypothesized that it would favorably alter these circulating biomarkers, as demonstrated in patients with HFrEF (11). The previous phase II PARAMOUNT (Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction) study in HFpEF lacked sufficient sample size to examine the effects of sacubitril/valsartan on these biomarkers. The PARAGON-HF (Prospective Comparison of ARNI With ARB Global Outcomes in HF With Preserved Ejection Fraction) trial provided this unique opportunity (12).

The following hypotheses were investigated in this analysis of PARAGON-HF: 1) circulating biomarkers that reflect the determinants of ECM homeostasis are abnormal in patients with HFpEF; 2) baseline values of these biomarkers and change from baseline predict HF events independently of clinical covariates and other biomarkers with established prognostic value (natriuretic peptides and troponin); and 3) treatment with sacubitril/valsartan, compared with valsartan

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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 *JACC* author instructions page.

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alone, favorably changes the levels of these biomarkers.

METHODS

STUDY DESIGN AND PROCEDURES. The design and primary results of the PARAGON-HF have been previously described (12,13). PARAGON-HF was a multicenter, double-blind clinical trial that randomized patients with chronic HFpEF in a 1:1 fashion to sacubitril/valsartan or valsartan alone. Eligibility criteria included signs and symptoms of HF, New York Heart Association functional classes II to IV, left ventricular ejection fraction (LVEF) ≥45%, age ≥50 years, diuretic therapy, evidence of structural heart disease, and elevated level of natriuretic peptides (with lower thresholds for patients with recent hospitalization for HF and higher thresholds in the presence of atrial fibrillation). Only patients who tolerated both study drugs during sequential run-in periods were eligible for randomization.

The pre-specified primary endpoint was a composite of total (first and recurrent) hospitalizations for HF and cardiovascular (CV) death. Key secondary endpoints included components of the primary endpoint. A blinded clinical events committee at Brigham and Women's Hospital (Boston, Massachusetts) adjudicated these endpoints.

PATIENT POPULATION. Patients recruited at selected sites were invited to participate in the collagen biomarker substudy. Most sites enrolling in this substudy were in North America or Europe; few centers in Asia and Latin America participated for logistical reasons. Five biomarkers were measured in 1,135 patients at the pre-specified baseline time point, after screening but prior to the start of the valsartan run-in period. Of these participants, 1,113 had a second measurement 16 weeks after randomization, and 1,016 had a third measurement 48 weeks after randomization. Analyses of patients at 16 and 48 weeks after randomization included only patients with available data at earlier time points as well.

BIOMARKERS. Five biomarkers of ECM homeostasis—sST2, PINP, PIIINP, TIMP-1, and CITP—were prospectively selected for measurement by the trial's executive committee and sponsor. Concentrations of these biomarkers were measured centrally at Clinical Reference Laboratory (Lenexa, Kansas) from frozen samples collected at individual sites. Samples were stored at −80°C. TIMP-1 was assayed in plasma and sST2, PINP, PIIINP, and CITP in serum. Commercial enzyme-linked immunosorbent assays for TIMP-1 (Quantikine, R&D Systems, Minneapolis, Minnesota) and sST2 (Presage, Critical Diagnostics, San Diego, California), and radioimmunoassays for PINP, PIIINP, and CITP (Orion Diagnostica, Espoo, Finland) were used. The coefficients of variance, lower limit of detection or lower limit of quantitation, and measuring range for each biomarker were previously published (11).

STATISTICAL ANALYSIS. Baseline biomarker levels were described using median (interquartile range) and compared qualitatively with referent control values (5,7,14–17) as previously described (11). Baseline characteristics of the included patients were described using proportions for categorical variables, mean ± SD for normally distributed continuous variables, and median and interquartile range for skewed continuous variables and were compared using Pearson chi-square test, Student's *t*-test, and Wilcoxon rank sum test, respectively. Changes in biomarker levels 16 and 48 weeks after randomization, compared to baseline, were described using geometric mean with 95% confidence intervals (CIs) for each treatment group; only patients with available data at all 3 time points were included in assessments of 48-week change. Biomarker changes from baseline were compared between treatment groups using linear regression with log-transformed biomarker values as the outcome and adjusting for log-transformed baseline biomarker level.

The relationships among log-transformed and baseline levels and incident rates of subsequent clinical outcomes were modeled using restricted cubic splines with 3 knots; log-transformed effects were reported per SD. These biomarkers were evaluated individually and together in recurrent events regression models using the method of Lin et al. (18) adjusted for 23 clinical covariates: age; sex; region; history of diabetes; hypertension; history of stroke; history of myocardial infarction; ischemic cause of HF; New York Heart Association functional class; prior HF hospitalization; angiotensin-converting enzyme inhibitor and/or angiotensin receptor blocker; mineralocorticoid antagonist; diuretic; beta-blocker; atrial fibrillation at screening; body mass index; LVEF; systolic blood pressure; diastolic blood pressure; serum potassium; estimated glomerular filtration rate; high-sensitivity troponin T (hsTnT); and N-terminal pro-B-type natriuretic peptide (NT-proBNP). The association between log-transformed and biomarker changes from baseline to 16 weeks and subsequent clinical outcomes were assessed using landmark analysis, both with and without adjustment for age, sex, New York Heart Association functional class, body mass index, estimated glomerular filtration rate, NT-proBNP, and hsTNT.

TABLE 1 Baseline Characteristics of Patients With Available Biomarker Data

	Sacubitril/Valsartan (n = 576)	Valsartan (n = 559)	p Value
Age, yrs	73.5 ± 7.8	73.6 ± 8.3	0.82
Female	292 (50.7)	294 (52.6)	0.52
Race			0.60
White	536 (93.1)	516 (92.3)	
Asian	16 (2.8)	21 (3.8)	
Black	7 (1.2)	8 (1.4)	
Other	17 (3.0)	14 (2.5)	
Region			0.67
Asia/Pacific	51 (8.9)	57 (10.2)	
Central Europe	274 (47.6)	264 (47.2)	
Latin America	9 (1.6)	4 (0.7)	
North America	70 (12.2)	68 (12.2)	
Western Europe	172 (29.9)	166 (29.7)	
Diabetes	246 (42.7)	225 (40.3)	0.40
Stroke	77 (13.5)	47 (8.4)	0.007
Hypertension	561 (97.4)	529 (94.6)	0.017
Prior myocardial infarction	125 (21.7)	117 (20.9)	0.75
Ischemic etiology	218 (37.8)	199 (35.6)	0.43
NYHA functional class			0.11
I	18 (3.1)	14 (2.5)	
II	449 (78.0)	419 (75.0)	
III	109 (18.9)	125 (22.4)	
IV	0 (0.0)	1 (0.2)	
Prior HF hospitalization	203 (35.2)	206 (36.9)	0.57
Body mass index, kg/m^2	30.7 ± 4.7	30.7 ± 4.9	0.94
Left ventricular ejection fraction	57.1 ± 7.6	57.4 ± 7.5	0.47
ACE inhibitor/ARB	511 (88.7)	499 (89.3)	0.77
Mineralocorticoid antagonist	137 (23.8)	139 (24.9)	0.67
Diuretic	553 (96.0)	546 (97.7)	0.11
Beta-blocker	469 (81.4)	466 (83.4)	0.39
Atrial fibrillation	185 (32.1)	177 (31.8)	0.9
Systolic blood pressure, mm Hg	131.1 ± 15.6	130.7 ± 15.1	0.65
Diastolic blood pressure, mm Hg	74.8 ± 10.7	74.0 ± 9.9	0.21
Potassium, mEq/l	4.5 ± 0.4	4.5 ± 0.4	0.91
eGFR, ml/min/1.73 m^2	60.2 ± 17.4	60.1 ± 18.4	0.91
NT-proBNP, pg/ml	941.5 (525.0–1,497.0)	953.0 (497.0–1,610.0)	0.77
hsTnT, ng/l	17.0 (12.0–26.0)	17.0 (11.0–26.0)	0.54

Values are mean ± SD, n (%), or median (interquartile range).

ACE = angiotensin-converting enzyme; ARB = angiotensin-receptor blocker; eGFR = estimated glomerular filtration rate; HF = heart failure; hsTnT = high-sensitivity troponin T; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association.

TABLE 2 Baseline Biomarker Data

	PARAGON-HF	Referent Controls*	Percentage Above Referent Control Median
sST2, ng/ml	24 (19–29)	20 (17–26)	69
TIMP-1, ng/ml	129 (108–156)	72 (70–75)	99
PINP, ng/ml	38 (29–51)	30 (25–35)	70
PIIINP, ng/ml	4.4 (3.6–5.5)	3.5 (3.0–4.0)	76
CITP, ng/ml	6.0 (4.7–8.0)	3.8 (2.8–5.3)	90

Values are median (interquartile range) unless otherwise indicated. *Referent controls are drawn from references 5, 7, and 14 to 17 as previously described (11).

CITP = collagen I telopeptide; IQR = interquartile range; PARAGON-HF = Prospective Comparison of ARNI With ARB Global Outcomes in HF With Preserved Ejection Fraction; PINP = N-terminal propeptide of collagen I; PIIINP = N-terminal propeptide of collagen III; sST2 = soluble ST2; TIMP = tissue inhibitor of matrix metalloproteinase 1.

Effect modification by randomized therapy was assessed via interaction terms. Similar time-to-first event Cox regression models were used for the endpoint of CV death.

All participants in PARAGON-HF provided written informed consent. The study protocols were approved by local ethics committees and institutional review boards at each participating site. Statistical analysis was performed in STATA version 14.1 (StataCorp, College Station, Texas). A 2-sided p value of <0.05 was considered significant.

RESULTS

BASELINE CHARACTERISTICS. The baseline characteristics of the study population are presented in Table 1 and Supplemental Table 1. Patients with available biomarker data, compared with patients without available data, were less likely to be Asian, on average 1 year older, with higher body mass index, lower estimated glomerular filtration rate, and lower rate of prevalent HF hospitalization in the past 9 months (all p < 0.001). Included patients also had a higher frequency of angiotensin-converting enzyme inhibitor or angiotensin-receptor blocker, diuretic, and beta-blocker use at baseline. Within the biomarker substudy, baseline characteristics of patients randomized to sacubitril/valsartan and valsartan alone were balanced (Table 1). Two parameters had clinically minor but statistically significant differences: patients randomized to sacubitril/valsartan had greater prevalence of prior stroke (14% vs. 8%; p = 0.01) and hypertension (97% vs. 95%; p = 0.02).

BASELINE BIOMARKER VALUES VERSUS REFERENT CONTROL SUBJECTS. Table 2 compares the baseline values of biomarkers in patients enrolled in PARAGON-HF with published values of these biomarkers from referent control subjects. Compared with referent control subjects, patients with HFpEF had greater median sST2, TIMP-1, PINP, PIIINP, and CITP. TIMP-1 and CITP exceeded referent control median values in >90% of patients.

Biomarker levels differed by sex: sST2, PIIINP, TIMP-1, and CITP were higher in men, whereas PINP was higher in women (Supplemental Table 2A). There were no statistically significant differences in baseline biomarker levels between patients with LVEF ≤57% or >57% (the median value) (Supplemental Table 2B).

Effects of treatment with sacubitril/valsartan versus valsartan on biomarkers. Figure 1 shows the effect of treatment with sacubitril/valsartan or valsartan on geometric mean biomarker levels 16 and 48 weeks after randomization. Sacubitril/valsartan decreased TIMP-1 by 8% (95% CI: 6% to 10%; $p < 0.001$), sST2 by 4% (95% CI: 1% to 7%; $p = 0.002$), and PIIINP by 3% (95% CI: 0% to 6%; $p = 0.04$) at 16 weeks, compared with valsartan after adjustment for baseline value (**Figures 1A and 1C**). CITP was increased by 4% (95% CI: 1% to 8%; $p = 0.02$) with sacubitril/valsartan compared with valsartan. There were no statistically significant differences in PINP between treatment groups.

At 48 weeks after randomization, reductions in TIMP-1 and sST2 with sacubitril/valsartan compared with valsartan were maintained (**Figure 1B**). PINP and PIIINP were numerically lower and CITP numerically higher with sacubitril/valsartan, but these differences were not statistically significant.

The effect of sacubitril/valsartan compared with valsartan on biomarker levels 16 weeks after randomization was similar in men and women and patients with lower versus higher LVEF (**Figure 2**), despite differences in primary endpoint treatment effect between these groups.

BASELINE BIOMARKER VALUES VERSUS OUTCOMES. The risk of the primary outcome (composite of CV death and total heart failure hospitalizations) increased with greater baseline values of TIMP-1, sST2, PIIINP, and CITP, but not PINP, in unadjusted analysis (**Table 3, Figures 3A and 3B**). After adjustment for 23 covariates including LVEF, NT-proBNP, hsTNT, and randomized treatment group, sST2 and TIMP-1 retained statically significant associations with the primary endpoint. When all biomarkers and clinical covariates were included in 1 model, only higher TIMP-1 concentration remained independently associated with risk of the primary endpoint. Thus, in these patients with HFpEF, baseline values of TIMP-1 predicted outcomes independently of the other 4 profibrotic biomarkers, NT-proBNP, and hsTNT. Associations of biomarker levels with the components of the primary endpoint, CV death and HF hospitalization, were consistent in direction and approximate magnitude (Supplemental Table 3), though only sST2 independently predicted CV death after multivariable adjustment due to lower statistical power.

Baseline biomarker levels did not modify the effect of sacubitril/valsartan compared with valsartan on the primary endpoint (p for interaction >0.05 for all).

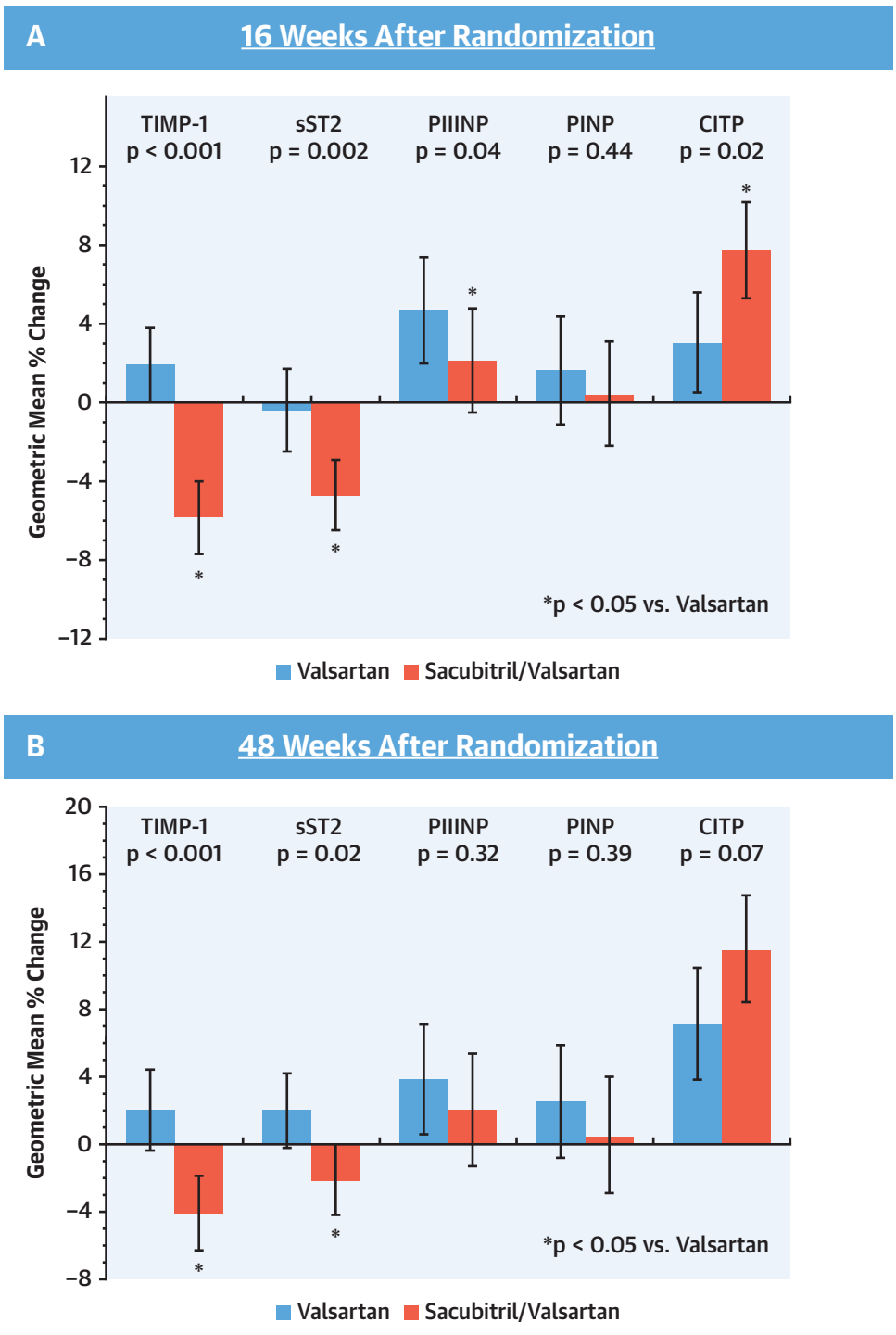
Relationship between change in biomarker from baseline and outcomes. Increases in biomarker levels from baseline to week 16 were associated with increased risk of subsequent primary endpoint events for all 5 biomarkers after adjustment for baseline value. After further adjustment for 7 covariates, changes in TIMP-1 (rate ratio [RR]: 1.23 per SD increase; 95% CI: 1.03 to 1.47; $p = 0.02$), sST2 (RR: 1.15 per SD increase; 95% CI: 1.02 to 1.31; $p = 0.03$), and CITP (RR: 1.26 per SD increase; 95% CI 1.03 to 1.55; $p = 0.03$) remained associated with the primary endpoint (Supplemental Table 4). The relationships between change in TIMP-1 and sST2 from baseline to 16 weeks after randomization and risk of subsequent primary endpoints are presented in **Figures 3C and 3D**.

DISCUSSION

Circulating biomarkers reflecting mechanisms of ECM homeostasis (sST2), collagen synthesis (PINP, PIIINP), and collagen degradation and turnover (TIMP-1, CITP) were abnormal in patients with HFpEF enrolled in the PARAGON-HF trial, suggesting that profibrotic signaling may play a role in the pathophysiology of HFpEF. Higher baseline levels of sST2 and TIMP-1 and increases in these markers over time were associated with greater risk of HF hospitalization and CV death. Sacubitril/valsartan altered these biomarkers in a pattern that suggests a decrease in profibrotic signaling. All of these findings were independent of sex and EF. In aggregate, these data suggest that biomarkers reflecting ECM homeostasis are elevated in HFpEF, altered by sacubitril/valsartan, and have important prognostic value (**Central Illustration**).

COMPARISON TO PUBLISHED STUDIES. Baseline values. Baseline values of the biomarkers examined in PARAGON-HF were comparable to HF cohorts derived from both epidemiologic studies and randomized clinical trials. TIMP-1 levels exceeded the referent control median in >90% of patients and were similar to patients with HFrEF in PARADIGM-HF (Prospective Comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure) (7,11,15). PIIINP and CITP levels exceeded referent controls and were similar to those of patients with HFpEF in the Cardiovascular Health Study (17). Elevation in PIIINP was similar to that of patients with HFpEF in I-PRESERVE (Irbesartan in

FIGURE 1 **Change in Biomarker Levels by Treatment Group at 16 and 48 Weeks After Randomization**



Change in biomarker levels by randomized treatment group 16 weeks **(A)** and 48 weeks **(B)** after randomization. **(B)** Only patients with available data at all 3 time points (baseline, week 16, and week 48) are included. **(C)** Geometric mean change in biomarker level with sacubitril/valsartan compared with valsartan, adjusted for baseline value, at 16 weeks. **Error bars** indicate 95% confidence interval. *p $<$ 0.05 versus valsartan. CITP = carboxyl-terminal telopeptide of collagen type I; PIIINP = N-terminal propeptide of collagen III; PINP = N-terminal propeptide of collagen I; Sac = sacubitril; sST2 = soluble ST2; TIMP = tissue inhibitor of matrix metalloproteinase; Val = valsartan.

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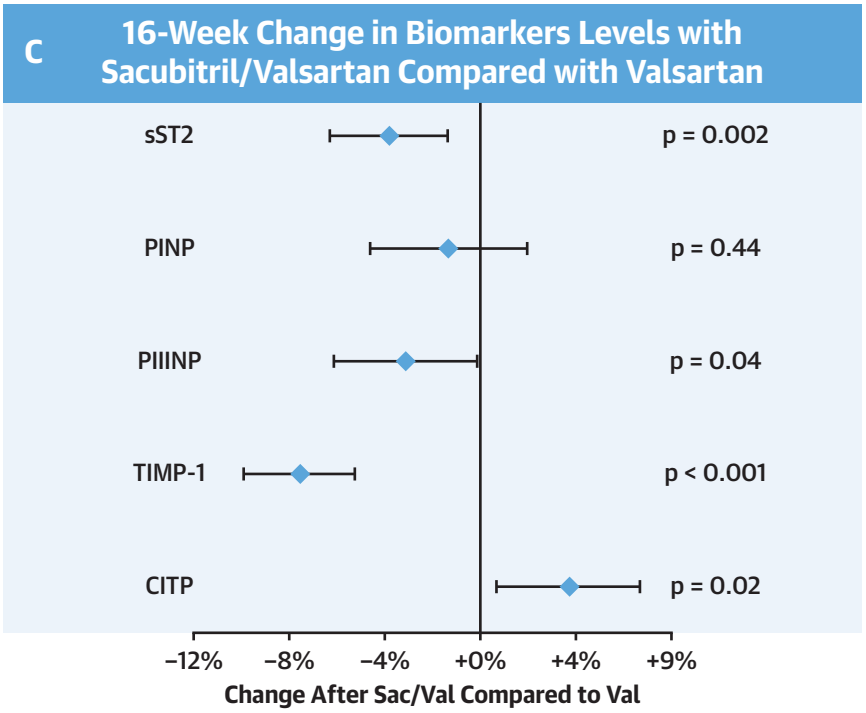
FIGURE 1 Continued



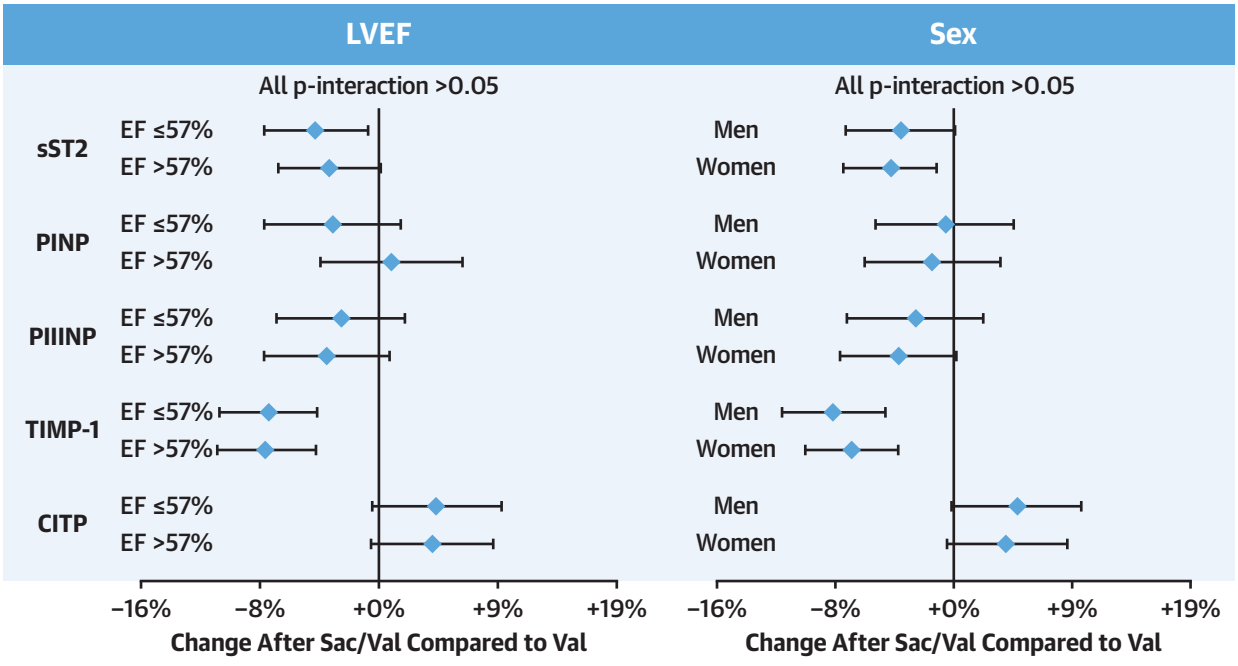
Heart Failure With Preserved Systolic Function) (19) and is the only marker studied with confirmed association to histologically proven myocardial fibrosis (20,21). sST2 levels exceeded subjects of similar age and sex in the Framingham Heart Study (14). PINP levels exceeded referent controls in the SHIP (Study of Health in Pomerania) study and were slightly lower than for patients with HFpEF in I-PRESERVE (19,22).

Relationship to outcome. In the current study, both baseline and change from baseline values of sST2 and TIMP-1 had a significant relationship with the primary study outcome of CV death and HF hospitalization. These results in this carefully defined HFpEF population are similar to cohorts of patients with HFrEF derived from epidemiologic studies and randomized clinical trials (11,23). However, to our knowledge, this is the first study to demonstrate this relationship in patients with HFpEF. The relationships between higher levels of sST2 and TIMP-1 (and increases in these markers) and greater risk of CV death and HF hospitalization remained statistically significant even after robust multivariable adjustment for all baseline covariates including NT-proBNP and hsTNT.

When all 5 measured biomarkers of ECM homeostasis were added to the multivariate model, TIMP-1 retained a significant relationship with the primary endpoint. Thus, TIMP-1 had prognostic significance independent of the other biomarkers of ECM homeostasis and natriuretic peptides and troponin. Similar associations for TIMP-1 were also observed in patients with HFrEF in the PARADIGM-HF trial (11). TIMP-1 was the strongest predictor of all-cause mortality after age in a cohort of >5,000 Icelandic patients (24), and in the Framingham Heart Study, TIMP-1 was associated with LV hypertrophy and systolic dysfunction by echocardiogram (25). TIMP-1 appears to identify patients with chronic HF at the highest risk for events, regardless of EF.

EFFECTS OF SACUBITRIL/VALSARTAN. The changes in biomarker levels observed with sacubitril/valsartan compared with valsartan alone would be predicted to reflect significant changes in ECM homeostasis. TIMP-1 inhibits a number of MMPs, thus inhibiting the catabolism of insoluble fibrillar collagen and promoting its accumulation. In PARAGON-HF and PARADIGM-HF, treatment with sacubitril/valsartan decreased TIMP-1; therefore sacubitril/valsartan may

FIGURE 2 Change in Biomarker Level by Treatment 16 Weeks After Randomization by Sex and LVEF



Change in biomarker level with sacubitril/valsartan versus valsartan 16 weeks after randomization in men and women and patients with lower and higher left ventricular ejection fraction (LVEF). Geometric mean change in biomarker level with sacubitril/valsartan compared with valsartan, adjusted for baseline value at 16 weeks. The median LVEF was 57%. Abbreviations as in Figure 1.

alter ECM homeostasis in a manner that may reduce profibrotic signaling. sST2 serves as a decoy for interleukin 33, facilitates profibrotic signaling through the ST2 receptor and may lead to increased collagen synthesis. In the PARAGON-HF, PARADIGM-HF, and PIONEER-HF (Comparison of Sacubitril/Valsartan Versus Enalapril on Effect on NT-proBNP in Patients Stabilized From an Acute Heart Failure Episode) trials, treatment with sacubitril/valsartan decreased sST-2 and therefore may alter ECM homeostasis in a manner that may reduce profibrotic and inflammatory signaling (11,26). The magnitude of TIMP-1 and sST2 reduction with sacubitril/valsartan was similar regardless of EF in this study and compared with reduction in patients with reduced EF in PARADIGM-HF.

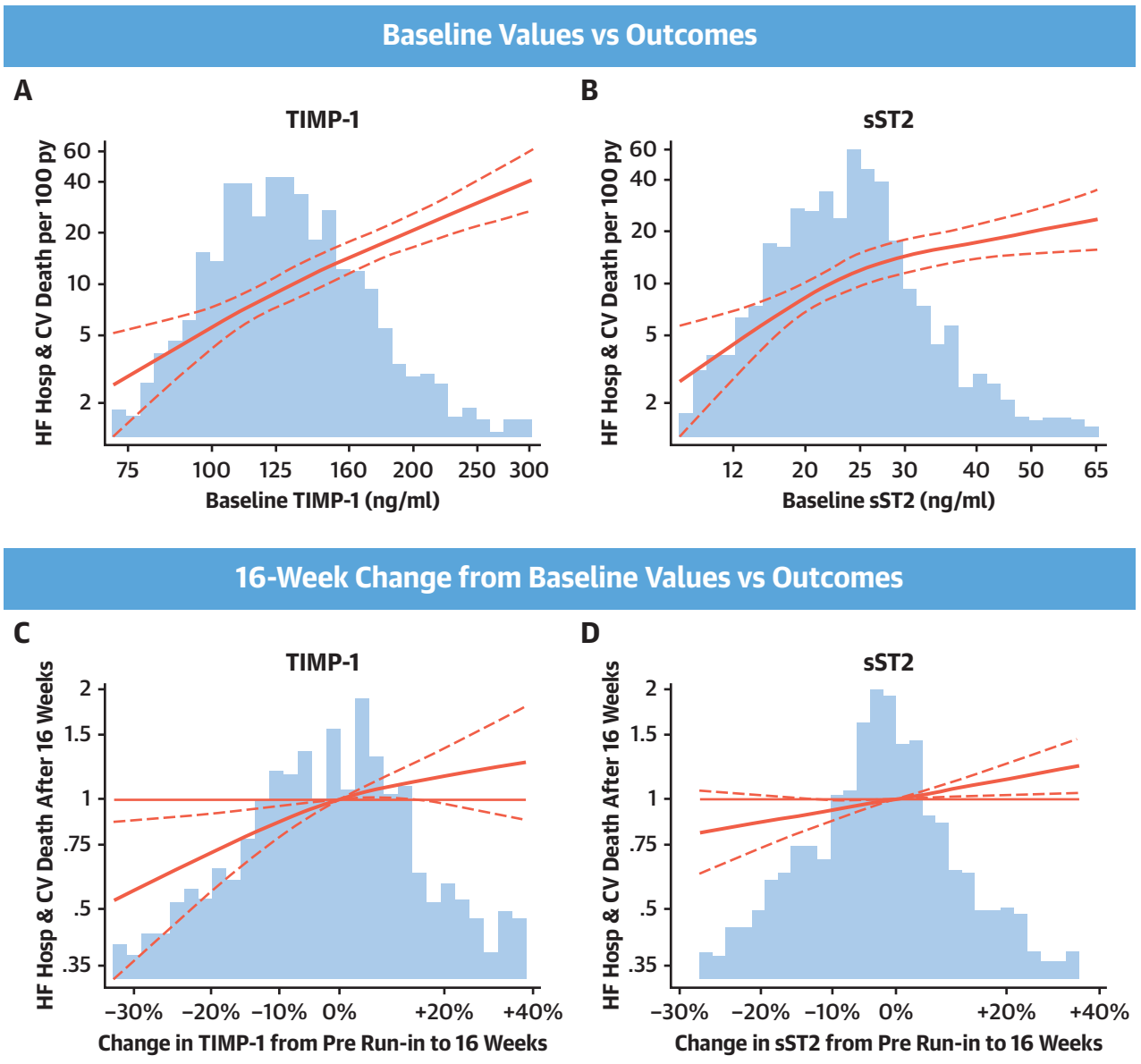
TABLE 3 Association Between Baseline Biomarker Levels and Primary Endpoint

		Unadjusted		Adjusted for Baseline Covariates		Adjusted for Baseline Covariates + Other 4 ECM Biomarkers	
	n	RR (95% CI) per SD	p Value	RR (95% CI) per SD	p Value	RRR (95% CI) per SD	p Value
TIMP-1	1,117	1.70 (1.49–1.94)	<0.001	1.36 (1.17–1.58)	<0.001	1.36 (1.15–1.61)	<0.001
sST2	1,120	1.43 (1.27–1.60)	<0.001	1.14 (1.01–1.29)	0.04	1.00 (0.87–1.15)	0.99
PIIINP	1,108	1.32 (1.12–1.56)	<0.001	1.04 (0.88–1.23)	0.65	1.05 (0.84–1.30)	0.69
PINP	1,116	1.06 (0.87–1.28)	0.56	0.92 (0.77–1.10)	0.34	0.85 (0.67–1.07)	0.16
CITP	1,096	1.55 (1.32–1.82)	<0.001	1.04 (0.86–1.26)	0.68	1.00 (0.80–1.25)	0.99

The primary endpoint was a composite of CV death and total HF hospitalizations (335 events). Rate ratio (RR) with 95% confidence interval (CI) refers to increase in expected event rate per SD increase in log-transformed biomarker level and was calculated using recurrent events regression analysis. The following covariates were included: NT-proBNP; high-sensitivity troponin; age; sex; region; history of stroke, diabetes, hypertension, myocardial infarction, ischemic cause of HF, or atrial fibrillation; NYHA functional class; left ventricular ejection fraction; medications (mineralocorticoid receptor antagonist, diuretic, beta-blocker); systolic and diastolic blood pressure; eGFR; and serum potassium.

ECM = extracellular matrix; other abbreviations as in Tables 1 and 2.

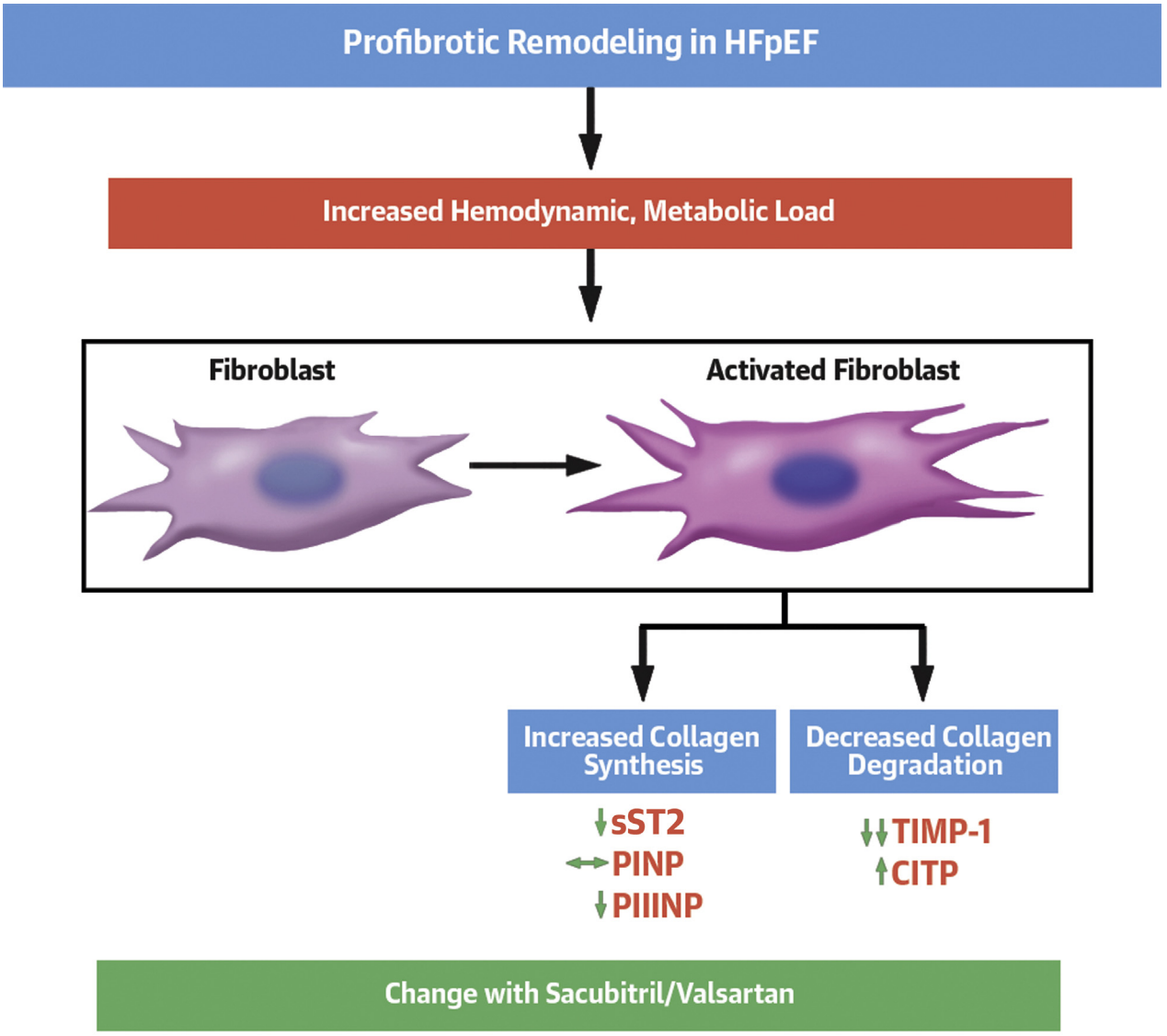
FIGURE 3 TIMP-1 and sST2 Baseline Values and 16-Week Change With Incidence of HF Hospitalization and CV Death



Continuous relationships of TIMP-1 and sST2 baseline values and 16-week change with incidence of subsequent heart failure (HF) hospitalization (Hosp) and cardiovascular (CV) death. **(A, B)** The x-axis and histogram represent plasma biomarker level at baseline. The **solid line** represents estimated incidence rate of the primary endpoint, total HF hospitalizations and CV death, without adjustment. The **dashed lines** represent 95% confidence intervals for the estimated incidence rate. Incidence rate is displayed on the primary (left-sided) y-axis. The highest and lowest 3% of biomarker values are not shown. **(C, D)** The x-axis and histogram represent change in biomarker level between pre-run-in baseline visit and the week 16 visit. The **solid line** represents estimated incidence rate of the primary endpoint, total HF hospitalizations and CV death, that occurred after 16 weeks, relative to patients with no change in biomarker level, adjusted for log-transformed baseline value. The **dashed lines** represent 95% confidence intervals for the estimated incidence rate. Incidence rate ratio is displayed on the primary (left-sided) y-axis. The highest and lowest 5% of biomarker change values are not shown. py = patient-years; other abbreviations as in Figure 1.

The increase in CITP with sacubitril/valsartan may appear inconsistent with reductions in TIMP-1 and sST2, as all 3 markers are postulated to associate with greater profibrotic signaling. One explanation is that chronic elevations in CITP reflect greater collagen turnover (which may adversely affect prognosis),

CENTRAL ILLUSTRATION **Fibrosis Biomarkers in Heart Failure With Preserved Ejection Fraction**



Cunningham, J.W. et al. J Am Coll Cardiol. 2020;76(5):503–14.

Increased hemodynamic and metabolic loads produced by the antecedent disease processes lead to the development of the clinical syndrome of heart failure with preserved ejection fraction (HFpEF). Activated fibroblasts alter both collagen synthesis and degradation; circulating biomarkers reflect these changes and are altered by treatment with sacubitril/valsartan. CITP = carboxyl-terminal telopeptide of collagen type I; PIIINP = N-terminal propeptide of collagen III; PINP = N-terminal propeptide of collagen I; sST2 = soluble ST2; TIMP = tissue inhibitor of matrix metalloproteinase.

whereas short-term changes are driven by collagen degradation (which may facilitate more normal ECM homeostasis). In chronic pathological states, collagen turnover increases to a higher steady state level. Existing normal collagen is degraded and replaced with newly synthesized collagen that is processed and cross-linked in an abnormal fashion. This increased collagen turnover would be characterized by increased propeptides and telopeptides, the pattern observed in this study and others, in which

patients with HFpEF had higher values of PINP, PIIINP, and CITP than did referent control subjects or patients with diabetes and hypertension but not heart failure (7,17,27). These clinical data are also supported by data from a recent porcine model of pressure-overload that results in HFpEF and abnormal ECM architecture (28). On the other hand, the short-term increase in CITP with sacubitril/valsartan may reflect greater collagen degradation, particularly when viewed in combination with a coordinate decrease in TIMP-1. In this substudy, sacubitril/valsartan decreased TIMP-1, which would be expected to allow increased MMP degradation of fibrillar collagen and the release of increased CITP. Moreover, although the increase in CITP cannot be dismissed, baseline CITP was not independently associated with the primary endpoint and the increase in CITP with sacubitril/valsartan was less statistically robust than decreases in TIMP-1 and sST2 and was observed only at 16 and not 48 weeks.

STUDY LIMITATIONS. Baseline biomarker values were compared with those of referent control subjects from previous publications, not with those of patients enrolled contemporaneously as part of PARAGON-HF. However, studies with similar assays and patient demographics were selected, except for the presence of HF. Plasma and serum biomarker concentrations may be influenced by processes outside the myocardium, such as renal or hepatic dysfunction, bone or skin disease, systemic inflammation, malignancy, or pregnancy. However, patients with these conditions were excluded from the PARAGON-HF trial. Comparison to the active comparator valsartan, which may itself have antifibrotic effects, may have resulted in smaller treatment effects than would have been observed with a placebo control. The assays for TIMP-1, PIIINP, and CITP are indicated for research use only; the assays for sST2 and PINP are approved for clinical use by the U.S. Food and Drug Administration.

CONCLUSIONS

Patients with HFpEF who were enrolled in the PARAGON-HF trial had elevated levels of circulating biomarkers reflecting abnormal ECM homeostasis. Baseline biomarker levels and short-term changes were associated with subsequent risk of HF events independently of clinical risk factors. Sacubitril/valsartan significantly altered these biomarkers. These data suggest that interstitial myocardial fibrosis contributes to HFpEF pathogenesis and prognosis and that antifibrotic properties may contribute to the mechanism of potential benefit of sacubitril/valsartan in patients with HFpEF.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Reductions in biomarkers of perturbed ECM homeostasis during treatment with sacubitril/valsartan are associated with improved clinical outcomes in patients with HFpEF.

TRANSLATION OUTLOOK: Further studies are warranted to understand the effects of sacubitril/valsartan on fibrotic signaling on the pathobiology of ECM homeostasis in patients with HFpEF.

REFERENCES

1. Kasner M, Westermann D, Lopez B, et al. Diastolic tissue Doppler indexes correlate with the degree of collagen expression and cross-linking in heart failure and normal ejection fraction. J Am Coll Cardiol 2011;57:977–85.

2. Zile MR, Baicu CF, Ikonomidis JS, et al. Myocardial stiffness in patients with heart failure and a preserved ejection fraction: contributions of collagen and titin. Circulation 2015;131:1247–59.

3. López B, Ravassa S, González A, et al. Myocardial collagen cross-linking is associated with heart failure hospitalization in patients with hypertensive heart failure. J Am Coll Cardiol 2016;67:251–60.

4. González A, Schelbert EB, Díez J, Butler J. Myocardial interstitial fibrosis in heart failure. biological and translational perspectives. J Am Coll Cardiol 2018;71:1696–706.

5. Duprez DA, Gross MD, Kizer JR, Ix JH, Hundley WG, Jacobs DR. Predictive value of collagen biomarkers for heart failure with and without preserved ejection fraction: MESA (Multi-Ethnic Study of Atherosclerosis). J Am Heart Assoc 2018;7:e007885.

6. Zile MR, Jhund PS, Baicu CF, et al., for the PARAMOUNT Investigators. 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. Circ Heart Fail 2016;9:e002551.

7. Zile MR, Desantis SM, Baicu CF, et al. Plasma biomarkers that reflect determinants of matrix composition identify the presence of left ventricular hypertrophy and diastolic heart failure. Circ Heart Fail 2011;4:246–56.

8. Zannad F, Alla F, Dousset B, Perez A, Pitt B, Rales Investigators. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the

Randomized Aldactone Evaluation Study (RALES). Circulation 2000;102:2700–6.

9. Iraqi W, Rossignol P, Angioi M, et al. Extracellular cardiac matrix biomarkers in patients with acute myocardial infarction complicated by left ventricular dysfunction and heart failure: insights from the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) study. Circulation 2009;119:2471–9.

10. Tamura N, Ogawa Y, Chusho H, et al. Cardiac fibrosis in mice lacking brain natriuretic peptide. Proc Natl Acad Sci U S A 2000;97:4239–44.

11. Zile MR, O'Meara E, Claggett B, et al. Effects of sacubitril/valsartan on biomarkers of extracellular matrix regulation in patients with HFrEF. J Am Coll Cardiol 2019;73:795–806.

12. Solomon SD, McMurray JJV, Anand IS, et al., for the PARAGON-HF Investigators and Committees. Angiotensin–neprilysin inhibition in heart failure with preserved ejection fraction. N Engl J Med 2019;381:1609–20.

13. Solomon SD, Rizkala AR, Gong J, et al. Angiotensin receptor neprilysin inhibition in heart failure with preserved ejection fraction: rationale and design of the PARAGON-HF trial. J Am Coll Cardiol HF 2017;5:471–82.

14. Coglianese EE, Larson MG, Vasan RS, et al. Distribution and clinical correlates of the interleukin receptor family member soluble ST2 in the Framingham Heart Study. Clin Chem 2012;58:1673–81.

15. Essa EM, Zile MR, Stroud RE, et al. Changes in plasma profiles of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in stress-induced cardiomyopathy. J Card Fail 2012;18:487–92.

16. Alla F, Kearney-Schwartz A, Radauceanu A, Dores SD, Dousset B, Zannad F. Early changes in serum markers of cardiac extra-cellular matrix turnover in patients with uncomplicated hypertension and type II diabetes. Eur J Heart Fail 2006;8:147–53.

17. Barasch E, Gottdiener JS, Aurigemma G, et al. The relationship between serum markers of collagen turnover and cardiovascular outcome in the elderly: the Cardiovascular Health Study. Circ Heart Fail 2011;4:733–9.

18. Lin DY, Wei LJ, Yang I, Ying Z. Semiparametric regression for the mean and rate functions of recurrent events. J Royal Stat Soc Ser B Stat Methodol 2000;62:711–30.

19. Krum H, Elsik M, Schneider HG, et al. Relation of peripheral collagen markers to death and hospitalization in patients with heart failure and preserved ejection fraction: results of the I-PRESERVE collagen substudy. Circ Heart Fail 2011;4:561–8.

20. Klappacher G, Franzen P, Haab D, et al. Measuring extracellular matrix turnover in the serum of patients with idiopathic or ischemic dilated cardiomyopathy and impact on diagnosis and prognosis. Am J Cardiol 1995;75:913–8.

21. López B, González A, Ravassa S, et al. Circulating biomarkers of myocardial fibrosis: the need for a reappraisal. J Am Coll Cardiol 2015;65:2449–56.

22. Michelsen J, Wallaschofski H, Friedrich N, et al. Reference intervals for serum concentrations of three bone turnover markers for men and women. Bone 2013;57:399–404.

23. Aimo A, Januzzi JL Jr., Bayes-Genis A, et al. Clinical and prognostic significance of sST2 in heart failure: JACC Review Topic of the Week. J Am Coll Cardiol 2019;74:2193–203.

24. LaRocca G, Aspelund T, Greve AM, et al. Fibrosis as measured by the biomarker, tissue inhibitor metalloproteinase-1, predicts mortality in Age Gene Environment Susceptibility-Reykjavik (AGES-Reykjavik) study. Eur Heart J 2017;38:3423–30.

25. Sundström J, Evans JC, Benjamin EJ, et al. Relations of plasma total TIMP-1 levels to cardiovascular risk factors and echocardiographic measures: the Framingham Heart Study. Eur Heart J 2004;25:1509–16.

26. Morrow DA, Velazquez EJ, DeVore AD, et al. Cardiovascular biomarkers in patients with acute decompensated heart failure randomized to sacubitril-valsartan or enalapril in the PIONEER-HF trial. Eur Heart J 2019;40:3345–52.

27. Zhang ZY, Ravassa S, Yang WY, et al. Diastolic left ventricular function in relation to urinary and serum collagen biomarkers in a general population. PLoS One 2016;11:e0167582.

28. Torres WM, Barlow SC, Moore A, et al. Changes in myocardial microstructure and mechanics with progressive left ventricular pressure overload. J Am Coll Cardiol Basic Trans Science 2020;5:463–80.

KEY WORDS biomarkers, fibrosis, heart failure

APPENDIX For supplemental tables, please see the online version of this paper.