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Effects of Sacubitril/Valsartan on Biomarkers of Extracellular Matrix Regulation in Patients With HFrEF

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ABSTRACT

BACKGROUND Myocardial fibrosis is an important pathophysiological mechanism underlying the development of heart failure (HF). Given the biochemical targets of sacubitril/valsartan, we hypothesized that circulating biomarkers reflecting the mechanisms that determine extracellular matrix (ECM) homeostasis, including collagen synthesis, processing, and degradation, are altered by sacubitril/valsartan in comparison to enalapril.

OBJECTIVES The purpose of this study was to examine the effects of sacubitril/valsartan on biomarkers of ECM homeostasis and the association between the rate of primary composite outcome (cardiovascular death or HF hospitalization) and these biomarkers.

METHODS Biomarkers at baseline (n = 2,067) and both baseline and 8 months after randomization (n = 1,776) included aldosterone, soluble ST2 (sST2), tissue inhibitor of matrix metalloproteinase (TIMP)-1, matrix metalloproteinase (MMP)-2, MMP-9, Galectin-3 (Gal-3), N-terminal propeptide of collagen I (PINP), and N-terminal propeptide of collagen III (PIIINP). The effects of sacubitril/valsartan on biomarkers were compared with enalapril. Baseline biomarker values and changes from baseline to 8 months were related to primary outcome.

RESULTS At baseline, the profibrotic biomarkers aldosterone, sST2, TIMP-1, Gal-3, PINP, and PIIINP were higher, and biomarkers associated with collagen degradation, MMP-2 and -9, were lower than published referent control values. Eight months after randomization, aldosterone, sST2, TIMP-1, MMP-9, PINP, and PIIINP had decreased more in the sacubitril/valsartan than enalapril group. At baseline, higher values of sST-2, TIMP-1, and PIIINP were associated with higher primary outcome rates. Changes from baseline to 8 months in sST-2 and TIMP-1 were associated with change in outcomes.

CONCLUSIONS Biomarkers associated with profibrotic signaling are altered in HF with reduced ejection fraction, sacubitril/valsartan significantly decreased many of these biomarkers, and these biomarkers have important prognostic value. These findings suggest that sacubitril/valsartan may reduce profibrotic signaling, which may contribute to the improved outcomes. (This Study Will Evaluate the Efficacy and Safety of LCZ696 Compared to Enalapril on Morbidity and Mortality of Patients With Chronic Heart Failure [PARADIGM-HF]; NCT01035255) (J Am Coll Cardiol 2019;73:795–806)

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ABBREVIATIONS AND ACRONYMS

BNP = B-type natriuretic peptide

Gal-3 = galectin-3

HFrEF = heart failure with reduced ejection fraction

hsTnT = high-sensitivity troponin T

MMP = matrix metalloproteinase

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

PIIINP = N-terminal propeptide of collagen III

sST2 = soluble ST-2

TIMP = tissue inhibitor of matrix metalloproteinase

Myocardial fibrosis is an important pathophysiological mechanism involved in the development and progression of chronic heart failure (CHF) (1–3). The extent and distribution of myocardial fibrosis is the aggregate result of the homeostatic processes that govern collagen metabolism (3–6). These include collagen synthesis, processing, and degradation. Collagen synthesis by myocardial fibroblasts is affected by hemodynamic, neurohumoral, metabolic, and other profibrotic and antifibrotic determinants that are activated in diseases such as CHF (4–6). For example, collagen synthesis by fibroblasts is increased by aldosterone, soluble ST-2 (sST-2), galectin-3 (Gal-3), and tissue inhibitor of matrix metalloproteinase (TIMP)-1. Each of these proteins/peptides are present in sufficient quantities to be measured in the plasma of referent control and CHF patients (3,7–9). Newly synthesized collagen must be processed by removing the C-terminal and N-terminal propeptides. The N-terminal propeptide of collagen I (PINP) and N-terminal propeptide of collagen III (PIIINP) can be measured in the serum of control and CHF patients (7–9). Collagen can be further processed (and degraded) by matrix metalloproteinases (MMPs) such as MMP-2 and -9.

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In a recent review, Ferreira et al. (10) summarized the few studies that have examined circulating biomarkers that reflect extracellular matrix (ECM) homeostasis in patients with heart failure and reduced ejection fraction (HFrEF) and that examined the relationship between these biomarkers and prognosis or response to therapy (10). Of these, only 4 studies, with a limited number of circulating biomarkers that reflect some aspect of ECM homeostasis, had sample sizes in excess of 200 subjects. These studies demonstrated variable prognostic value of collagen propeptides, collagen teleopeptides, and MMP-1 on clinical outcomes. MMP-1 and PIIINP had the most significant relationship to outcomes and were decreased with treatment with mineralocorticoid receptor antagonists (MRAs) and cardiac resynchronization therapy. However, these studies did not examine a comprehensive panel of biomarkers that represent determinants of ECM homeostasis, nor did they adjust these analyses for clinical/demographic parameters, other biomarkers with known prognostic value (natriuretic peptides and troponin), or other ECM homeostasis biomarkers.

The PARADIGM-HF (Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure trial) provided a unique opportunity to examine a panel of biomarkers that reflect ECM homeostasis in a large cohort of well-characterized HFrEF patients that included long-term outcome data. In the current analysis of PARADIGM-HF, the following hypotheses were examined: 1) circulating biomarkers that reflect the determinants of ECM homeostasis are abnormal in patients with HFrEF; 2) both baseline and change from baseline values in biomarkers that represent determinants of ECM homeostasis have incremental prognostic value after adjusting for clinical/demographic parameters and other biomarkers with known prognostic value (natriuretic peptides and troponin); and 3) treatment with sacubitril/valsartan leads to changes in these biomarkers that are compatible with an antifibrotic effect.

METHODS

STUDY DESIGN AND PROCEDURES. The design and primary results of PARADIGM-HF have been extensively described elsewhere (11–13). Patients with chronic HF, New York Heart Association functional class II to IV symptoms, elevated plasma levels of

and Theracos; and has served as a consultant for Akros, Alnylam, Amgen, AstraZeneca, Bayer, Bristol-Myers Squibb, Cardior, Corvia, Cytokinetics, Gilead, GlaxoSmithKline, Ironwood, Merck, Novartis, Roche, Takeda, Theracos, Quantum Genetics, Cardurion, AoBiome, Janssen, and Cardiac Dimensions. Drs. Solomon, Swedberg, Packer, McMurray, and Rouleau have participated in Executive Steering Committee activities for clinical studies sponsored by Novartis. Dr. Packer has served as a consultant for Akcea, Amgen, AstraZeneca, Boehringer Ingelheim, Cardiorentis, Daiichi-Sankyo, Novo Nordisk, Relypsa, Sanofi, and Synthetic Biologics. Dr. McMurray's employer, Glasgow University, has been paid by Novartis for his time spent as Executive Committee member and then co-principal investigator of ATMOSPHERE, co-principal investigator of the PARADIGM-HF and PARAGON-HF trials, and Executive/Steering Committee member for PARADISE-MI and PERSPECTIVE trials (with sacubitril/valsartan), and meetings/presentations related to these trials, aliskiren, and sacubitril/valsartan; his travel and accommodation for some of these meetings has also been paid by Novartis (these payments were made through a consultancy with Glasgow University, and Dr. McMurray has not received personal payments in relation to these trials/drugs). Dr. Rouleau has served as a consultant to Novartis and AstraZeneca. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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natriuretic peptides, and systolic left ventricular (LV) dysfunction (LV ejection fraction ≤40%) were eligible for randomization in PARADIGM-HF. The primary outcome was a composite of death from cardiovascular (CV) causes or a first hospitalization for heart failure. The relationship between these CV outcomes and 8 plasma biomarkers (described in the following text) was examined.

PATIENT POPULATION. For logistical reasons, centers in Asia/South Pacific and in South America did not participate in the biomarker ancillary study. Patients recruited at selected North American and European sites in the PARADIGM-HF trial were invited to participate in the biomarker study. A total of 2,067 participants were enrolled and had 8 biomarkers measured at baseline (before run-in). Of these participants, 1,776 had a second measurement of these biomarkers at 8 months after randomization (Table 1). Unless stated otherwise, all analyses of baseline data are obtained from the full cohort of 2,067 patients, while analyses of post-baseline biomarkers are obtained from the 1,776 patients with data available at both time points.

BIOMARKERS. Aldosterone, TIMP-1, MMP-2, and MMP-9 were assayed in plasma and sST2, Gal-3, PINP, and PIIINP in serum. Samples were collected, stored, and transferred to the central laboratory as previously described (13). The following assays were used: Aldosterone (DiaSorin Liaison assay, Saluggia, Italy), TIMP-1 (R&D Systems, Minneapolis, Minnesota), MMP-2 and -9 (Meso Scale Discovery, Gaithersburg, Maryland), Galectin-3 (BG Medicine, Waltham, Massachusetts), PINP and PIIINP (Orion Diagnostica, Espoo, Finland), sST2 (Critical Diagnostics Presage assay, San Diego, California). The coefficient of variance, lower limit of detection, or lower limit of quantitation, and measuring range for each biomarker are presented in Online Table 1. Analyses were adjusted for: B-type natriuretic peptide (BNP) (Siemens Centaur assay, Tarrytown, New York), N-terminal propeptide of B-type natriuretic peptide (NT-proBNP) (Roche Diagnostics, Indianapolis, Indiana) and high-sensitivity troponin T (hsTnT) (Roche Diagnostics GmbH, Mannheim, Germany).

Changes in biomarkers from baseline to 8 months were compared between treatment groups: enalapril and sacubitril/valsartan. Baseline values were related to the rate of primary outcome, CV death, and HF hospitalization for the trial as a whole. Biomarker changes from baseline to 8 months were related to the rate of primary outcome using a landmark analysis beginning after the 8-month time point.

TABLE 1 Baseline Demographics of Patients With Baseline and Follow-Up Biomarker Data

	Enalapril (n = 881)	Sacubitril/Valsartan (n = 895)	p Value
Age, yrs	67 ± 10	67 ± 10	0.97
Female	174 (20)	158 (18)	0.26
Body mass index, kg/m^2	29.8 ± 5.5	29.4 ± 5.5	0.18
NYHA functional class			0.94
I	21 (2)	19 (2)	
II	647 (74)	670 (75)	
III	206 (23)	201 (22)	
IV	5 (1)	5 (1)	
LV ejection fraction	31 ± 6	31 ± 6	0.78
Prior use of ACEi	688 (78)	739 (83)	0.018
Prior use of ARB	201 (23)	162 (18)	0.014
Prior HF hospitalization	523 (59)	524 (59)	0.73
Hypertension status	686 (78)	686 (77)	0.54
Race			0.49
White	843 (96)	857 (96)	
Black	24 (3)	20 (2)	
Asian	4 (0)	2 (0)	
Other	10 (1)	16 (2)	
Region			0.57
North America	131 (15)	145 (16)	
Latin America	0 (0)	0 (0)	
Western Europe and other	408 (46)	394 (44)	
Central Europe	342 (39)	356 (40)	
Asia-Pacific	0 (0)	0 (0)	
Systolic blood pressure	123 ± 16	124 ± 16	0.22
Diabetes mellitus	355 (40)	350 (39)	0.61
Heart rate	72 ± 12	71 ± 12	0.25
Ischemic cardiomyopathy	565 (64)	574 (64)	1.00
Prior myocardial infarction	422 (48)	444 (50)	0.47
Prior atrial fibrillation	440 (50)	424 (47)	0.28
Prior stroke	98 (11)	80 (9)	0.13
ICD	244 (28)	254 (28)	0.75
CRT	100 (11)	90 (10)	0.38
Diuretic agents	725 (82)	719 (80)	0.29
Beta-blockers	840 (95)	855 (96)	0.85
Digoxin	214 (24)	180 (20)	0.034
Aldosterone	423 (48)	375 (42)	0.010
Baseline creatinine	1.2 ± 0.3	1.2 ± 0.3	0.55
Baseline BNP, pg/ml	216 (148-378)	225 (150-392)	0.31
Baseline NT-proBNP, pg/ml	1,423 (822-2,756)	1457 (831-2,816)	0.62
hsTnT, ng/l	16 (10-25)	16 (10-24)	0.54

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

ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; BNP = B-type natriuretic peptide; CRT = cardiac resynchronization therapy; HF = heart failure; hsTnT = high-sensitivity troponin T; ICD = implantable cardioverter-defibrillator; LV = left ventricular; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association.

STATISTICAL ANALYSIS. Baseline biomarker data were compared qualitatively with referent control values (7,14-29). Referent control values were presented for comparison as median (interquartile range [IQR]).

Biomarker levels at baseline and 8-month post-randomization are displayed using median (IQR).

Baseline characteristics of PARADIGM-HF patients (with or without a biomarker measurements) were summarized using mean ± SD, median (IQR), or frequency and percentages, as appropriate, with comparisons between the 2 groups conducted using Student's *t*-test, Wilcoxon rank sum test, and Pearson's chi-square test, respectively. Biomarker values at baseline and 8 months post-randomization and corresponding changes from baseline were summarized for each treatment group using median (IQR), using quantile regression, adjusting for the baseline value, to compare the changes between treatment groups. Similarly, biomarker values were also summarized using geometric means, with percent changes from baseline compared using linear regression with log-transformed biomarker values as the outcome and adjusting for log-transformed baseline biomarker. The proportion of patients with biomarker levels that exceeded the referent control median value was reported and compared using unadjusted logistic regression. Additionally, the proportions of patients in each treatment group with a biomarker increase or decrease from baseline of specific magnitude were reported.

The relationships between baseline biomarkers and incident rates of subsequent clinical outcomes were assessed using restricted cubic spline models with 3 knots in models. These baseline relationships were adjusted using the following parameters applied individually or in combination: baseline covariates (defined in the following text), BNP and NT-proBNP, hsTnT, randomized treatment group (enalapril or sacubitril/valsartan), and baseline values of all 8 fibrosis-related biomarkers (aldosterone, TIMP-1, MMP-2, MMP-9, sST2, Gal-3, PINP, and PIIINP). In addition, for the change from baseline analyses, baseline values of each biomarker were also used for adjustments. Baseline characteristics listed in **Table 1** (baseline covariates) included: age; sex; geographic region; body mass index; New York Heart Association functional class; LV ejection fraction; prior hospitalization for heart failure; hypertension; diabetes; ischemic etiology; prior myocardial infarction; atrial fibrillation; heart rate; systolic blood pressure; creatinine; prior stroke; implantable cardioverter-defibrillator; cardiac resynchronization therapy; or prior use of an angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, diuretics, beta-blockers, digoxin, or mineralocorticoid receptor antagonist.

Previous studies using the PARADIGM-HF study population have demonstrated that baseline covariates, BNP, NT-proBNP, hsTnT, and randomized treatment group were predictive of patient outcomes. Therefore, all of these parameters were used to adjust the analytic models that examined the relationship between the 8 profibrotic biomarkers and outcomes. These are the primary analyses presented in the results in the following text. The predictive value that an individual profibrotic biomarker added as a baseline value to these models was also examined as a secondary analysis using a C-statistic model described in the following text. The change from baseline to 8 months after randomization of all 8 fibrosis-related biomarkers was not added as a covariate adjustment to the analysis examining the relationship between change from baseline and outcomes, because the number of events that occurred after the landmark time was limited and would not support a robust statistical analysis.

Adjusted hazard ratios were produced using Cox proportional hazards models using both untransformed and log-transformed biomarkers. Landmark analyses of proportional changes in biomarkers from baseline to month 8 versus subsequent clinical outcomes were assessed using adjusted Cox proportional hazards models. All events that occurred between baseline and 8 months were excluded from this landmark analysis examining events subsequent to 8 months. Effect modification by randomized therapy was assessed via interaction terms for all clinical outcomes. Harrell's C-statistics for models with and without 5 sets of covariate conditions were tested for both the primary endpoint and CV mortality: baseline covariates (M1); M1 plus BNP, NT-proBNP, and hsTnT (M2); M2 plus TIMP-1 (M3); M2 plus all 8 profibrotic biomarkers (M4); and M4 minus M1 (M5).

All analyses were conducted using STATA version 14 (College Station, Texas). All p values <0.05 were considered to be statistically significant, and no adjustments were made for multiple comparisons.

RESULTS

BASELINE CHARACTERISTICS AND OUTCOMES. The characteristics of the study population are presented in **Table 1** and in Online Tables 2 and 3. Patients who participated in the biomarker study and patients who did not participate in the biomarker study had several statistically significant differences; all analyses were adjusted for each of the characteristics listed in **Table 1** that differed significantly between the 2 groups. Within the biomarker study group, those randomized to enalapril versus sacubitril/valsartan had very few differences in any of the baseline parameters listed in **Table 1**. Three parameters had clinically minor but statistically significant differences. Compared with patients taking enalapril,

patients taking sacubitril/valsartan had less prior use of an MRA (48% vs. 42%; p = 0.008), less prior angiotensin receptor blocker use (22% vs. 18%; p = 0.015), and more prior angiotensin-converting enzyme inhibitor use (79% vs. 83%; p = 0.019).

BASELINE BIOMARKER VALUES VERSUS REFERENT CONTROL SUBJECTS. Table 2 shows the comparison of the baseline values of biomarkers in patients enrolled in PARADIGM-HF with published values of these biomarkers from referent control subjects. This analysis showed that, compared with referent control subjects, patients with HFrEF had increased aldosterone, sST2, Gal-3, TIMP-1, PINP, and PIIINP and decreased MMP-2 and -9. The percent of the PARADIGM-HF patients that had values of aldosterone, sST2, Gal-3, TIMP-1, PINP, and PIIINP greater than the referent control median and values of MMP-2 and -9 below the referent control median were >65% for all 8 biomarkers, and >85% for 4 of the biomarkers.

EFFECTS OF TREATMENT WITH SACUBITRIL/VALSARTAN VERSUS ENALAPRIL ON BIOMARKERS. Figure 1 graphically displays the effects of treatment with enalapril versus sacubitril/valsartan on the geometric mean of biomarkers from baseline value to 8 months after randomization expressed as percent change. Aldosterone, sST2, MMP-9, TIMP-1, and PINP were significantly reduced with sacubitril/valsartan compared with enalapril treatment (all comparisons p < 0.05 sacubitril/valsartan vs. enalapril) adjusted for baseline biomarker values. Compared with enalapril, sacubitril/valsartan treatment decreased aldosterone by −6% (95% confidence interval [CI]: −11% to −1%), sST2: −7% (95% CI: −9% to −4%), MMP-9: −8% (95% CI: −14% to −2%), TIMP-1: −4% (95% CI: −7% to −1%), and PINP: −6% (95% CI: −10% to −3%). There were no statistically significant differences between treatment groups with respect to changes in MMP-2, galectin-3, and PIIINP.

An additional analysis was performed in which baseline systolic blood pressure and change in systolic blood pressure was used as a covariate in analyses that compared changes in biomarkers in the 2 treatment groups. These analyses suggested that the differential effect of sacubitril/valsartan on the profibrotic biomarkers were independent of the change in blood pressure. These data are presented in Online Table 4.

BASELINE BIOMARKER VALUES VERSUS OUTCOMES. The relationships between baseline values of the 8 profibrotic biomarkers, the risk of the primary outcome (combination of CV death and HF hospitalization) and the risk of CV death alone are presented in Figure 2 and Table 3. The higher the baseline value of sST2, TIMP-1, or PIIINP, the higher the subsequent rates of CV death and HF hospitalizations. These relationships were statistically significant after adjustment for baseline covariates, the biomarkers BNP and NT-proBNP, hsTnT, and randomized treatment group. By contrast, there were no relationships between outcomes and aldosterone, MMP-2, MMP-9, Gal-3, or PINP.

Next, we addressed the issue of whether any individual profibrotic biomarker added prognostic value independent of the other profibrotic biomarkers and independent of baseline covariates, BNP,

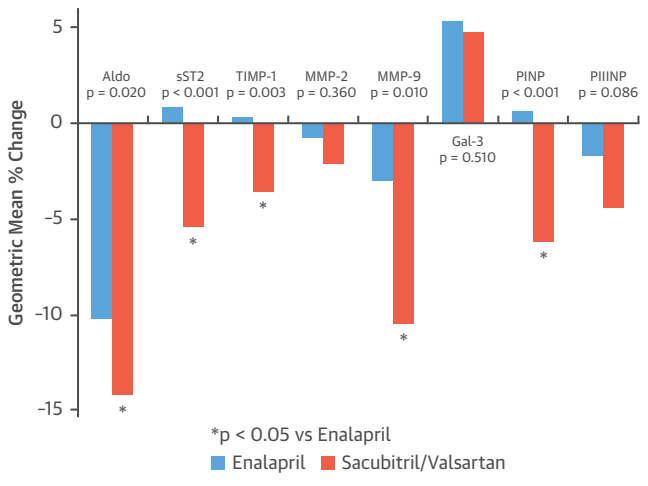
TABLE 2 Baseline Biomarker Data

Marker	PARADIGM-HF	Referent Controls	Patients Above/Below Referent Control Median
Aldosterone, pmol/l	275 (174–465)	200 (150–225)	↑ 68
sST2, ng/ml	32 (25–42)	20 (17–26)	↑ 92
Galectin-3, ng/ml	17 (14–21)	12 (9–15)	↑ 88
MMP-2, ng/ml	135 (117–158)	335 (323–443)	↓ 97
MMP-9, ng/ml	64 (38–126)	95 (90–110)	↓ 66
TIMP-1, ng/ml	125 (105–152)	72 (70–75)	↑ 99
PINP, ng/ml	36 (27–48)	30 (25–35)	↑ 65
PIIINP, ng/ml	4.6 (3.6–5.9)	3.5 (3.0–4.0)	↑ 78

Values are median (interquartile range) or %. ↑ and ↓ represent the directional differences between PARADIGM-HF patients and mean referent control values and the numerical value of that change.

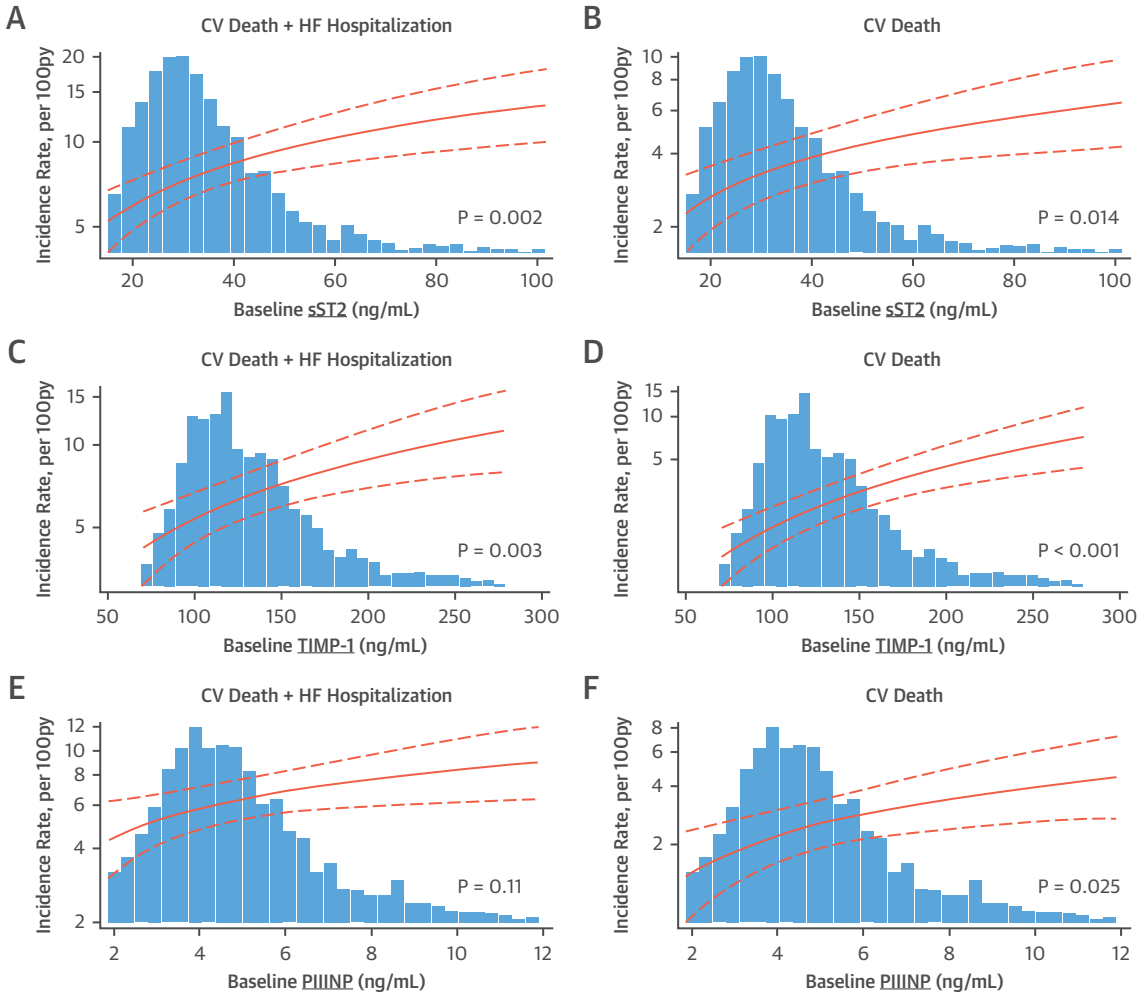
MMP = matrix metalloproteinase; PARADIGM-HF = Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in HF; PIIINP = N-terminal propeptide of collagen III; PINP = N-terminal propeptide of collagen I; sST2 = soluble ST-2; TIMP = tissue inhibitor of matrix metalloproteinase.

FIGURE 1 Effects of Treatment on the Geometric Mean Percent Change in Biomarkers From Baseline to 8 Months After Randomization



Sacubitril/valsartan treatment **(orange bars)** was associated with a significantly larger decrease in aldosterone (aldo), soluble ST2 (sST2), matrix metalloproteinase (MMP)-9, tissue inhibitor of matrix metalloproteinase (TIMP)-1, and N-terminal propeptide of collagen I (PINP) than treatment with enalapril **(blue bars)**. *p < 0.05 versus enalapril.

FIGURE 2 Baseline Biomarker Versus Outcome



The risk of the primary outcome (combination of cardiovascular [CV] death and heart failure [HF] hospitalization) and the risk of CV death alone increased with increasing baseline values of sST2 **(A and B)**, TIMP-1 **(C and D)**, and N-terminal propeptide of collagen III (PIIINP) **(E and F)**. The higher the baseline value of sST2, TIMP-1, or PIIINP, the higher the subsequent rates of CV death and HF hospitalizations. These relationships were significant after adjustment for treatment and baseline covariates including B-type natriuretic peptide, N-terminal pro–B-type natriuretic peptide, and high-sensitivity troponin T. py = person years; other abbreviations as in Figure 1.

NT-proBNP, hsTnT, and randomized treatment group using a C-statistical analysis. Online Table 5 shows the relationship between baseline values of each of the 8 profibrotic biomarkers and the primary outcome and CV death after adjustment of the model for all 8 biomarkers. TIMP-1 had prognostic significance independent of the other 7 biomarkers and the other adjustment variables. For TIMP-1, the HR for the primary outcome was 1.20 (95% CI: 1.03 to 1.38; p = 0.017) and for CV death was 1.43 (95% CI: 1.14 to 1.79; p = 0.002). Thus, in these patients with HFrEF, baseline values of TIMP-1, independent of the other 7 profibrotic biomarkers and independent of BNP, NT-proBNP, and hsTnT, predicted patient outcomes. Using sequential modeling and a C-statistical method, the additional prognostic significance of each adjustment parameter was quantified (Online Table 6). Again, of all 8 profibrotic biomarkers, only TIMP-1 improved the C-statistic.

RELATIONSHIP BETWEEN CHANGE IN BIOMARKER FROM BASELINE AND OUTCOMES. The relationships between change from baseline to 8 months after randomization of the 8 profibrotic biomarkers, the risk of the primary outcome (composite of CV death or HF hospitalization), and the risk of CV death alone are

TABLE 3 Relationships Between Biomarkers and Outcomes

			Baseline Levels vs. Outcomes HR (95% CI), p Value Log-Transformed, per SD				8-Month Changes vs. Subsequent Outcomes HR (95% CI), p Value, per 20% Increase			
Marker	Visit	Median [IQR]	Primary Outcome*	p Value	CV Death*	p Value	Primary Outcome†	p Value	CV Death†	p Value
Aldo, pmol/l	Baseline	275 [173–464]	0.96 (0.87–1.07)	0.47	1.00 (0.87–1.16)	0.97	0.97 (0.93–1.01)	0.16	0.97 (0.92–1.02)	0.27
	M8	243 [154–394]								
sST2, ng/ml	Baseline	32.2 [25.4–41.5]	1.16 (1.06–1.28)	0.002	1.18 (1.03–1.35)	0.014	1.14 (1.06–1.23)	< 0.001	1.05 (0.96–1.16)	0.28
	M8	31.0 [24.7–39.3]								
TIMP-1, ng/ml	Baseline	125 [106–152]	1.21 (1.07–1.37)	0.003	1.55 (1.28–1.87)	<0.001	1.03 (0.94–1.13)	0.50	1.19 (1.05–1.35)	0.006
	M8	123 [102–152]								
MMP-2, ng/ml	Baseline	135 [117–158]	1.07 (0.96–1.20)	0.24	1.06 (0.90–1.26)	0.49	1.03 (0.94–1.12)	0.54	1.03 (0.92–1.15)	0.62
	M8	133 [114–153]								
MMP-9, ng/ml	Baseline	64.1 [38.2–126.2]	1.02 (0.92–1.14)	0.66	0.99 (0.85–1.16)	0.94	1.00 (0.97–1.03)	0.93	0.99 (0.95–1.04)	0.79
	M8	59.3 [35.7–109.2]								
Galectin-3, ng/ml	Baseline	17.1 [13.9–21.2]	1.05 (0.95–1.16)	0.35	1.04 (0.91–1.20)	0.54	1.09 (1.00–1.20)	0.06	1.08 (0.96–1.22)	0.18
	M8	17.9 [14.4–22.3]								
PINP, ng/ml	Baseline	36.0 [27.0–48.0]	0.98 (0.87–1.11)	0.76	1.04 (0.87–1.24)	0.67	1.01 (0.95–1.08)	0.67	1.03 (0.95–1.13)	0.48
	M8	34.5 [25.5–46.5]								
PIIINP, ng/ml	Baseline	4.7 [3.6–5.9]	1.11 (0.98–1.26)	0.11	1.24 (1.03–1.49)	0.025	1.05 (0.97–1.13)	0.22	1.04 (0.95–1.14)	0.39
	M8	4.5 [3.6–5.8]								

Baseline covariates include age, sex, geographic region, body mass index, New York Heart Association functional class, left ventricular ejection fraction, prior heart failure hospitalization, hypertension, diabetes, ischemic etiology, prior myocardial infarction, atrial fibrillation, heart rate, systolic blood pressure, creatinine, prior stroke, implantable cardioverter-defibrillator, cardiac resynchronization therapy, prior use of an angiotensin-converting enzyme inhibitor, prior use of an angiotensin receptor blocker, diuretics, beta-blockers, digoxin, mineralocorticoid receptor antagonist, log(NT-proBNP), BNP, log(hsTnT), and randomized treatment (enalapril or sacubitril/valsartan). There were no statistically significant treatment interactions (all $p > 0.05$). *Adjusted for treatment + baseline covariates (including log-transformed BNP, NT-proBNP, and hsTnT). †Adjusted for treatment + baseline covariates (including log-transformed BNP, NT-proBNP, hsTnT) + baseline biomarker value.

IQR = interquartile range; other abbreviations as in Tables 1 and 2.

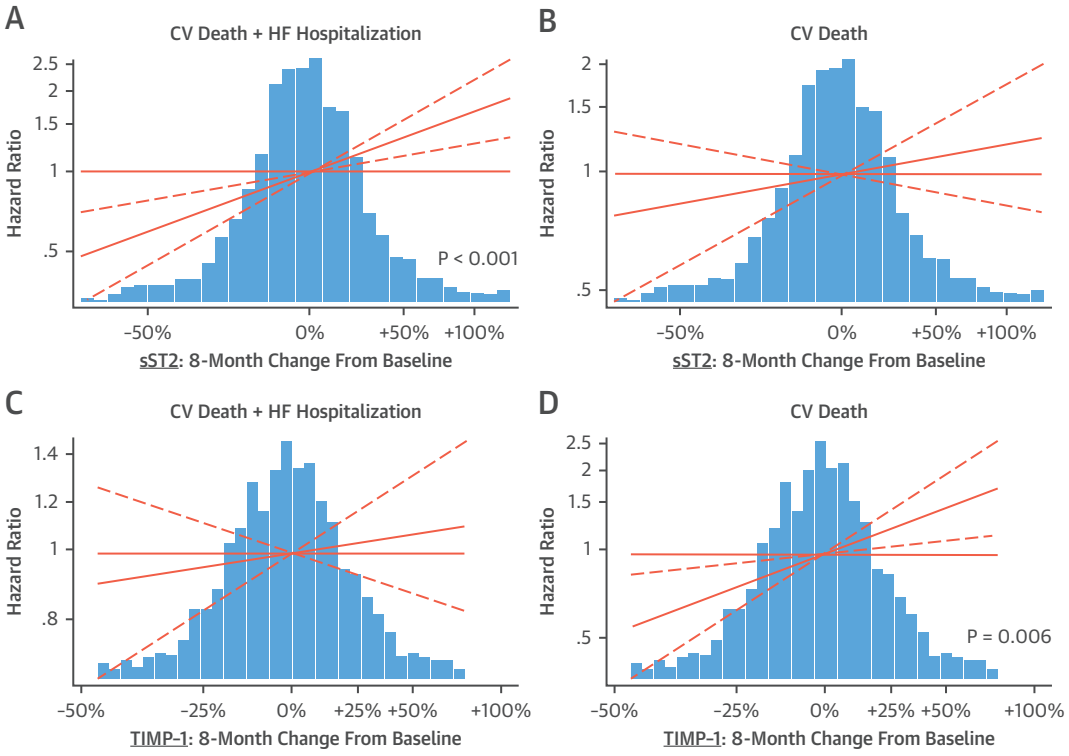
presented in Figure 3 and Table 3. The greater the decrease from baseline value of sST2 the greater the reduction in the subsequent rates of the primary outcome. The greater the decrease from baseline value of TIMP-1, the greater the reduction in the subsequent rates of CV death. These relationships were statistically significant after adjustment for baseline covariates, BNP, NT-proBNP, hsTnT, and randomized treatment group. By contrast, there were no relationships between outcomes and aldosterone, MMP-2, MMP-9, Gal-3, PINP, or PIIINP.

DISCUSSION

Biomarker data presented in the current analysis of the PARADIGM-HF study support 4 conclusions. First, biomarkers that reflect mechanisms of ECM homeostasis (aldosterone, sST2, TIMP-1, MMP-2, MMP-9, and Gal-3) and collagen synthesis (PINP and PIIINP) are altered in patients with HFrEF indicating the presence of profibrotic signaling. Second, treatment with sacubitril/valsartan alters these biomarkers of ECM homeostasis, decreasing determinants of collagen synthesis and processing, suggesting a decrease in the profibrotic state. Third, there is a significant relationship between baseline values of sST-2, TIMP-1, PIIINP, and the rate of primary composite outcome (CV death or HF hospitalization) in PARADIGM-HF patients. Fourth, there is a significant relationship between a change from baseline to 8 months after randomization values of sST-2, TIMP-1, and the rate of CV outcomes in PARADIGM-HF patients. In aggregate, these data suggest that 1 mechanism by which sacubitril/valsartan may exert a beneficial outcome in HFrEF patients may be related to a reduction in profibrotic signaling. The current study raises the possibility that further addition of biomarkers that reflect determinants of ECM homeostasis might improve these prognostic models. Clearly, however, additional studies must be performed.

IMPORTANCE OF MYOCARDIAL FIBROSIS IN HFrEF PATIENTS. Both replacement/reparative fibrosis, which replaces foci of necrotic or apoptotic cardiomyocytes, and reactive fibrosis, which occurs in response to increased metabolic and hemodynamic load, are 2 processes that may contribute to the structural and functional cardiac changes seen in patients with HFrEF (1,2,4). In HFrEF, these structural changes are associated with abnormalities in both systolic and diastolic function, may increase the propensity to arrhythmias (both atrial and ventricular), and may alter myocardial perfusion (30–32). The presence and extent of fibrosis has been shown to be associated with changes in morbidity and mortality rates in patients with heart failure (1,2,33).

FIGURE 3 Biomarker Change From Baseline Versus Outcome



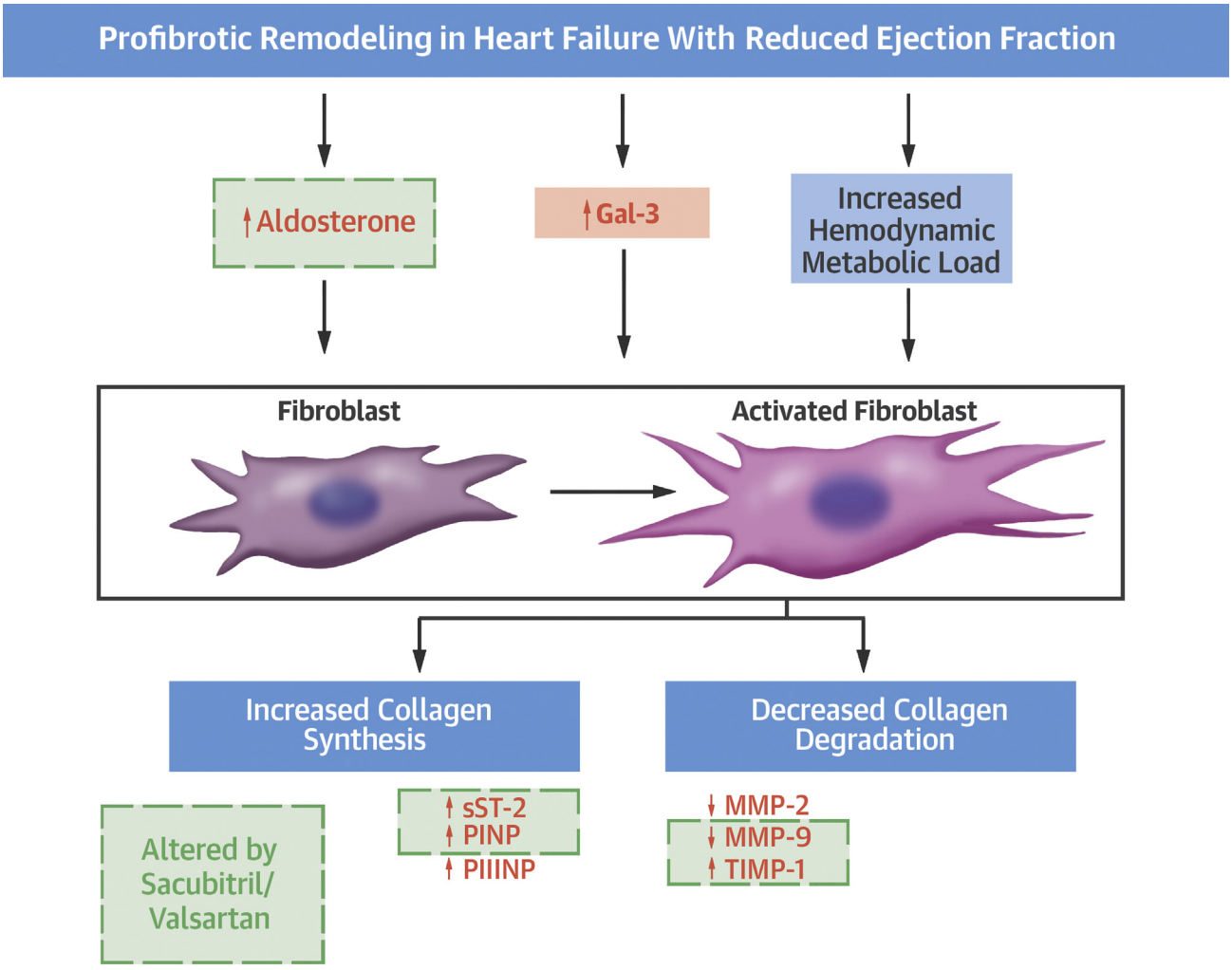
(A) Change in sST2 from baseline to 8 months after randomization was associated with a significant change in CV death and HF hospitalization after adjustment for treatment; baseline covariates including B-type natriuretic peptide (BNP), N-terminal proBNP, and high-sensitivity troponin T; and baseline value of sST2. There was a trend in the relationship between change in sST2 and CV death, but this did not reach statistical significance **(B)**. **(D)** Change in TIMP-1 from baseline to 8 months after randomization was associated with a change in CV death after adjustment for treatment; baseline covariates including BNP, N-terminal proBNP, and high-sensitivity troponin T; and baseline value of TIMP-1; there was a trend in the relationship between change in sST2 and CV death, but this did not reach statistical significance **(C)**. Abbreviations as in Figures 1 and 2.

Under some clinical circumstances, regression of fibrosis may be associated with lower morbidity and mortality. Examples include HFrEF patients who are treated with MRAs (34,35), patients with aortic valve stenosis who undergo aortic valve replacement, and patients with HFrEF who undergo left ventricular assist device implantation (36–39). Therefore, the ability to noninvasively assess the presence and extent of the profibrotic state, and treatment-induced changes in this state, using circulating biomarkers may have clinical application.

Although it is not possible at this point to directly measure collagen volume fraction using circulating biomarkers, it is possible to measure biomarkers that reflect changes in the determinants of ECM homeostasis. In myocardial samples from both patients with heart failure and animal models of heart failure, a correlation between circulating biomarkers and collagen volume fraction have been found (1,3,10). These correlative data support the utility of measuring circulating biomarkers.

BIOMARKERS THAT REFLECT MECHANISMS OF ECM HOMEOSTASIS. The homeostatic processes that govern ECM collagen metabolism include determinants of collagen synthesis, processing, cross-linking, and degradation (4). Collagen synthesis by myocardial fibroblasts is affected by hemodynamic, neurohumoral, metabolic and other profibrotic and antifibrotic determinants (**Central Illustration**). For example, collagen synthesis by fibroblasts (and possibly the activation of fibroblasts) can be induced by increased aldosterone, Gal-3, and increased hemodynamic and metabolic load. However, additional steps after collagen synthesis must occur before myocardial fibrosis develops. For example,

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



Zile, M.R. et al. J Am Coll Cardiol. 2019;73(7):795–806.

Biomarkers that reflect determinants of a profibrotic state in heart failure with reduced ejection fraction (HFrEF) and the effects of sacubitril/valsartan on these biomarkers. In patients with HFrEF, increased aldosterone, galectin-3, and metabolic/hemodynamic load activate fibroblasts to increase collagen synthesis as evidenced by increased PINP, PIIINP, and sST2. Activated fibroblasts secrete proteins and peptides that reduce collagen degradation, such as decreased MMP-2 and MMP-9 and increased TIMP-1. The changes in these biomarkers that reflect determinants of profibrotic state are indicated in **red**. Treatment with sacubitril/valsartan alters these biomarkers that reflect determinants of profibrotic state. Sacubitril/valsartan decreased aldosterone, sST2, MMP-9, TIMP-1, and PINP. Changes in sacubitril/valsartan-treated patients are indicated by **green dashed boxes**. MMP = matrix metalloproteinase; PIIINP = N-terminal propeptide of collagen III; PINP = N-terminal propeptide of collagen I; sST2 = soluble ST-2; TIMP = tissue inhibitor of matrix metalloproteinase.

newly synthesized collagen must be processed by removing the C-terminal and N-terminal propeptides and then cross-linked before it can form a structural insoluble collagen fiber. PINP and PIIINP can be measured in the plasma of control and HF patients, and when increased, indicate an increase in synthesis rate. Structural insoluble collagen fibers can be further processed (and degraded) by matrix metalloproteinases such as MMP-2 and -9. The activity of MMPs are further modulated by the endogenous inhibitors of MMPs, the TIMPs, such as TIMP-1. A number of MMPs and TIMPs secreted by myocardial fibroblasts can be measured in the circulation and have been found to be altered in heart failure patients; when MMPs are decreased and/or TIMPs are increased, there is a decrease in collagen degradation and an increase in collagen content. Thus, the extent and distribution of myocardial fibrosis, results from

the balance between collagen synthesis, processing, and degradation.

However, while individual biomarkers have been studied in HFrEF, a comprehensive examination of all of these profibrotic biomarkers, simultaneously, in a large group of HFrEF patients has not been performed before. Importantly, it has not been previously possible to relate biomarkers to prognosis, and to comprehensively examine the effects of treatment on a full range of markers of ECM homeostasis. Analyses from the current study addressed both of these issues.

BIOMARKERS AND PROGNOSIS. A large number of prognostic models have been developed in HFrEF and have been recently reviewed (40–42). In addition, biomarkers such as BNP, NT-proBNP, and hsTnT have been shown to provide additional prognostic value to these clinical risk scores. The current study suggests that the further addition of biomarkers that reflect determinants of ECM homeostasis may improve these prognostic models. In the current study, 3 profibrotic biomarkers had significant prognostic importance: sST2, TIMP-1, and PIIINP in a fashion that was independent of clinical parameters, natriuretic peptides, and troponin T and treatment effects. Furthermore, TIMP-1 had prognostic significance independent of the other 7 biomarkers. Thus, in this patient population of HFrEF, baseline values of TIMP-1, independent of the other 7 profibrotic biomarkers and independent of BNP, NT-proBNP, and hsTnT, predicted patient outcomes.

EFFECTS OF SACUBITRIL/VALSARTAN ON BIOMARKERS. Previous studies using PARADIGM-HF data showed that treatment with sacubitril/valsartan decreased NT-proBNP and hsTnT, but had no effect on GDF-15 (13,43). However, the current study is the first to show that sacubitril/valsartan alters a panel of profibrotic biomarkers that reflect changes in determinants of collagen synthesis, processing, and degradation. In addition, these effects were independent of changes in clinical parameters, BNP, NT-proBNP, and hsTnT. To date the only other drug that has been shown to alter any profibrotic biomarkers were the MRAs (both spironolactone and eplerenone), which decreased PIIINP. No other large cohort of well-characterized HFrEF patients coupled with long-term outcome data has shown the effects of drug therapy on a reasonably comprehensive panel of profibrotic biomarkers.

STUDY LIMITATIONS. The referent control data were assembled from "historic controls" aggregated from previous publications, and were not contemporaneous or obtained from an enrolled referent control cohort as part of PARADIGM-HF. Although this is certainly a limitation, the validity of the comparisons made between PARADIGM-HF patients and referent control subjects is supported by the extensive review (Online Table 7), the similarity in assay techniques, and similarity on demographics of referent subjects with respect to age, sex, and comorbidities, but the absence of heart failure.

Circulating biomarkers were measured using plasma or serum peripheral venous samples. Therefore, the myocardium, particularly the LV myocardium, is only one potential source for the proteins/peptides that were measured. However, the exclusion criteria used in PARADIGM-HF served to minimize the impact of most of the other potential organ sources of these biomarkers. For example, renal function was limited to those with modest reductions in estimated glomerular filtration rate, patients with chronic hepatic, bone, or skin disease, systemic inflammatory diseases, malignancies, and pregnancy were excluded. Under these circumstances, measured biomarkers may be substantially influenced by myocardial sources. Similar approaches have been used in many other prospective studies.

Given the exploratory nature of this analysis, multiple comparisons were made without formal adjustment for the number of biomarkers, outcomes, and time points under consideration. As such, type-I errors may be present, although we note that many reported results would remain significant at alpha = 0.006, reflecting a Bonferroni correction (0.05/8) for the number of biomarkers reported.

CONCLUSIONS

Biomarkers that reflect mechanisms of ECM homeostasis and collagen synthesis are altered in patients with HFrEF in a profibrotic manner. Baseline and change from baseline values of biomarkers associated with profibrotic signaling have important prognostic value. Sacubitril/valsartan significantly decreased these biomarkers. In aggregate, these data suggest that 1 mechanism by which sacubitril/valsartan may exert a beneficial outcome in HFrEF patients may be related to processes associated with changes in these biomarkers.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Improvement in morbidity and mortality during treatment with sacubitril/valsartan in patients with HFrEF may be related to inhibition of profibrotic signaling in the myocardium.

TRANSLATIONAL OUTLOOK: Further studies are needed to examine changes in ECM homeostasis and collagen synthesis and processing in patients with HFrEF during treatment with angiotensin receptor-neprolysin inhibition.

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KEY WORDS biomarkers, fibrosis, heart failure

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