Biomarkers that reflect extracellular matrix regulatory mechanisms in HFrEF patients: Effects of sacubitril/valsartan

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Abstract

Background: Myocardial fibrosis is an important pathophysiologic 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: Examine the effects of sacubitril/valsartan on biomarkers of ECM homeostasis and the association between the rate of primary composite outcome (CV death or HF hospitalization) and these biomarkers.

Methods: Biomarkers at baseline (2067 patients) and both baseline and 8 months after randomization (1776 patients) included aldosterone, sST2, TIMP-1, MMP-2, MMP-9, Gal-3, PINP, and 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, profibrotic biomarkers aldosterone, sST2, TIMP-1, Gal-3, PINP, PIIINP were higher and biomarkers associated with collagen degradation, MMP-2, MMP-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 a change in outcomes.

Conclusions: Biomarkers associated with profibrotic signaling are altered in HFrEF; 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.

Word Count: 250

Condensed Abstract

We tested the hypothesis that circulating biomarkers that reflect mechanisms of extracellular matrix homeostasis would be abnormal in patients with HFrEF enrolled in the PARADIGM-HF trial, have prognostic value, and reflect the therapeutic effect of sacubitril/valsartan. Biomarkers associated with profibrotic signaling are altered in HFrEF and sacubitril/valsartan significantly decreased these biomarkers. Baseline and change in biomarkers associated with profibrotic signaling have important prognostic value. These findings suggest that sacubitril/valsartan may reduce profibrotic signaling which may contribute to the improved outcomes.

Word Count: 72 words

Key Words: Heart failure, Biomarkers, Fibrosis

Abbreviations

HFrEF- Heart failure with a reduced ejection fraction

sST2- soluble ST-2

TIMP- Tissue inhibitor of matrix metalloproteinase

MMP- matrix metalloproteinase

Gal-3 – Galectin-3

PINP- n-terminal propeptide of collagen I

PIIINP- n-terminal propeptide of collagen III

CV- cardiovascular

COV - coefficient of variance

LOD - lower limit of detection

LLOQ - lower limit of quantitation

hsTnT – high sensitivity tropinin

HFH – herat failure hospitalization

ICD - implantable cardiac defibrillator

CRT - cardiac resynchronization therapy

LV = left ventricular

HF = heart failure

ACEi = angiotensin converting enzyme inhibitor

ARB = angiotensin receptor blocker

BNP = b-type natriuretic peptide

NT-proBNP = n-terminal pro BNP

NYHA = New York Heart Association

Twitter Account: @UofGICAMS

Twitter message: Biomarkers that reflect ECM homeostasis/fibrosis are abnormal in HFrEF patients, have prognostic value, and are altered by sacubitril/valsartan.

144 characters including spaces.

Introduction

Myocardial fibrosis is an important pathophysiologic 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 anti-fibrotic 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 1 (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 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 (MMP) such as MMP-2 and -9.

In a recent review, Ferreira et. al. summarized the few studies that have examined circulating biomarkers that reflect extracellular matrix (ECM) homeostasis in patients with heart failure and a reduced ejection fraction (HFrEF) and that examined the relationship between these biomarkers and prognosis or response to therapy (10). Of these, only four 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 Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure trial (PARADIGM-HF) 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); 3) treatment with sacubitril/valsartan leads to changes in these biomarkers that are compatible with an anti-fibrotic 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, NYHA class II–IV symptoms; elevated plasma levels of natriuretic peptides, and systolic LV dysfunction (LVEF ≤ 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 (HFH). The relationship between these CV outcomes and eight plasma biomarkers (described below) 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 2067 participants were enrolled and had 8 biomarkers measured at baseline (prior to run-in). Of these participants, 1776 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 2067 patients, while analyses of post-baseline biomarkers are obtained from the 1776 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 lab as previously described (13). The following assays were used: Aldosterone (DiaSorin Liaison assay,

Saluggia, Italy); TIMP-1 (R&D Systems, Minneapolis, MN), MMP-2 and MMP-9 (Meso Scale Discovery, Gaithersburg, MD), Galectin-3 (BG Medicine , Waltham, MA ; PINP, PIIINP (Orion Diagnostica, Espoo, Finland); Soluble ST2 (Critical Diagnostics Presage® assay, San Diego, CA). The coefficient of variance (COV), lower limit of detection (LOD), or lower limit of quantitation (LLOQ) and measuring range for each biomarker are presented in appendix Table 1. Analyses were adjusted for: B-type natriuretic peptide (BNP; Siemens Centaur assay, Tarrytown, NY), N-terminal propeptide of BNP (NT-proBNP; Roche Diagnostics, Indianapolis, IN) and high sensitivity troponin T (hs-TnT; 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 eighth month time point.

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 ranges [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 ± standard deviation, median (IQR), or frequency and percentages, as appropriate, with comparisons between the two groups conducted using t-test, Wilcoxon rank-sum test, and Pearson's chi-squared test, respectively. Biomarker values at baseline and month 8 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, biomarkers 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 three knots in models. These baseline relationships were adjusted using the following parameters applied individually or in combination: baseline covariates (defined below); BNP and NT-proBNP; hs-TnT; 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 (NYHA) class, left ventricular ejection fraction (LVEF), prior HFH, hypertension, diabetes, ischemic etiology, prior myocardial infarction, atrial fibrillation, heart rate, systolic blood pressure, creatinine, prior stroke, implantable cardioverter defibrillator (ICD), cardiac resynchronization therapy (CRT), prior use of an angiotensin converting enzyme inhibitor (ACEI), prior use of an angiotensin receptor blocker (ARB), diuretics, beta-blockers, digoxin, mineralocorticoid receptor antagonist (MRA).

Previous studies using the PARADIGM-HF study population have demonstrated that baseline covariates, BNP, NT-proBNP, hs-TnT 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 below. 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 below. 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 were 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 vs subsequent clinical outcomes were assessed using adjusted Cox proportional hazards models. All events that occurred between baseline and month 8 were excluded from this landmark analysis examining events subsequent to month 8. 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 hs-TnT (M2); M2 plus TIMP-1 (M3), M2 plus all 8 profibrotic biomarkers (M4), M4 minus M1 (M5).

All analyses were conducted using STATA version 14 (College Station, TX). 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, appendix tables 5 & 6. 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 vs 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 a MRA (48% vs. 42%, p=0.008), less prior ARB use (22% vs. 18%, p=0.015), more prior ACEi use (79% vs. 83%, p=0.019).

Baseline Biomarker Values vs Referent Controls

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 to referent controls, patients with HFrEF had increased aldosterone, sST2, Galectin-3, TIMP-1, PINP, PIIINP and decreased MMP-2 and -9. The percent of the PARADIGM-HF patients that had values of aldosterone, sST2, Galectin-3, TIMP-1, PINP, PIIINP greater than the referent control median and values of MMP-2 and -9 below the referent control median were more than 65% for all 8 biomarkers; > 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 signficantly reduced with sacubitril/valsartan compared with enalapril treatment (all comparisons $p < 0.05$ sacubitril/valsartan vs. enalapril) adjusted for baseline biomarker values. Compared to enalapril, sacubitril/valsartan treatment decreased aldosterone by -6% (95% CI -11% to -1%), sST2: -7% (-9% to -4%), MMP-9: -8% (-14% to -2%), TIMP-1: -4% (-7% to -1%), PINP: -6% (-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 two 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 Appendix Table 7.

Baseline Biomarker Values vs 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, NT-proBNP; hs-TnT 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, NT-proBNP; hs-TnT; and randomized treatment group using a C-statistical analysis. Supplemental Appendix Table 3 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 (95% CI) for the primary outcome was 1.20 (1.03-1.38) p=0.017 and for CV death was 1.43 (1.14-1.79) 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 hs-TnT, predicted patient outcomes. Using sequential modeling and a C-statistical method, the additional prognostic significance of each adjustment parameter was quantified (Supplemental Appendix Table 4). 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 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, hs-TnT, 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 four conclusions. First, biomarkers that reflect mechanisms of ECM homeostasis (aldosterone, sST2, TIMP-1, MMP-2, MMP-9, Gal-3) and collagen synthesis (PINP, 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 cardiovascular outcomes in PARADIGM-HF patients. In aggregate, these data suggest that one 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 two 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). Under some clinical circumstances, regression of fibrosis may is associated with lower morbidity and mortality. Examples include HFrEF patients that are treated with MRAs (34,35), patients with aortic valve stenosis that undergo aortic valve replacement , and patients with HFrEF that 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.

While 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 anti-fibrotic determinants (Figure 4). 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, 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 MMP's 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 hs-TnT 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, three 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 seven biomarkers. Thus, in this patient population of HFrEF, baseline values of TIMP-1, independent of the other seven profibrotic biomarkers and independent of BNP, NT-proBNP and hs-TnT, 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 HS troponin T, 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 the 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 hs-TnT. To date the only other drug that has been shown to alter any profibrotic biomarkers were the MRAs (both spirololactone 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.

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. While this is certainly a limitation, the validity of the comparisons made between PARADIGM-HF patients and referent controls is supported by the extensive review

(Appendix Table 2), the similarity in assay techniques, and similarity on demographics of referent subjects with respect to age, gender, co-morbidities 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 eGFR, 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, though 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 one mechanism by which sacubitril/valsartan may exert a beneficial outcome in HFrEF patients may be related to processes associated with changes in these biomarkers.

Perspectives

Clinical Competencies

Competency in Medical Knowledge: Myocardial fibrosis is an important pathophysiologic mechanism underlying the development of and the degree of illness in patients with heart failure and a reduced ejection fraction (HFrEF).

Competency in Patient Care: The appropriate use of sacubitril/valsartan in patients with HFrEF will result in a significant reduction in patient morbidity and mortality and these outcomes may be related to a change in the profibrotic signaling present in patients with HFrEF.

Translational Outlook implications:

Translational Outlook 1: The data presented in this manuscript examining the utility of profibrotic biomarkers will facilitate the development of new strategies for the management and treatment of patients with HFrEF.

Translational Outlook 2: The data presented in this manuscript examining the changes in profibrotic biomarkers that result from pharmacologic treatment and their resultant effects on morbidity and mortality markedly improve the understanding of the mechanism of action of sacubitril/valsartan.

Clinical Perspectives:

No previous analysis has been able to address the following critical clinically important issues:

1- Are plasma biomarkers that reflect mechanisms of extracellular matrix (ECM) homeostasis (aldosterone, sST2, TIMP-1, MMP-2, MMP-9 and Gal-3), collagen synthesis and processing (PINP, PIIINP) ("profibrotic biomarkers"), abnormal in patients with HFrEF?

2- Does treatment of HFrEF patients with sacubitril/valsartan reduce profibrotic biomarkers more than treatment with enalapril?

3) Is there a relationship between baseline values and change from baseline values of profibrotic biomarkers and a change in the morbidity and mortality in patients with HFrEF?

The answers to these questions have an important and practical influence on our understanding of CHF pathophysiology, use of biomarkers to facilitate treatment, and our understanding of the mechanism of action of sacubitril/valsartan.

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Figure Legends

Figure 1: Effects of treatment with enalapril (red bars) versus sacubitril / valsartan (green bars) on the geometric mean percent change in biomarkers from baseline to 8 months after randomization. * = $p < 0.05$ versus enalapril. Sacubitril / valsartan treatment was associated with a significantly larger decrease in aldosterone (aldo), soluble ST2 (sST2) matrix metalloproteinase (MMP)-9, tissue inhibitor of MMP (TIMP) -1, and n-terminal propeptide of collagen I (PINP) than treatment with enalapril.

Figure 2: Baseline Biomarker vs 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 soluble ST2 (sST2) (Panels A&B), tissue inhibitor of matrix metalloproteinase (TIMP)-1(Panels C&D), and n-terminal propeptide of collagen III (PIIINP) (Panels E&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 BNP, NT-proBNP and hsTnT.

Figure 3: Biomarker Change from Baseline vs Outcome.

Panel 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 BNP, NT-proBNP and hsTnT, 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 (Panel B). Panel 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, NT-proBNP and hsTnT, 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 (Panel C).

Central Illustration Figure 4: Biomarkers that reflect determinants of a profibrotic state in heart failure with a 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 9 and increased TIMP-1. The changes in these biomarkers that reflect determinants of profibrotic state are indicated in red type. 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 / valasartan treated patients are indicated by green dashed boxes.

Table 1: Baseline Demographics

Patients with baseline and follow-up biomarker data	**Enalapril**	**Sacubitril/valsartan**	
	n=881	**n=895**	**p-value**
Age (years)	67 ± 10	67 ± 10	0.97
Female sex	174 (20 %)	158 (18 %)	0.26
Body Mass Index	29.8 ± 5.5	29.4 ± 5.5	0.18
NYHA Class			0.94
1	21 (2 %)	19 (2 %)	
2	647 (74 %)	670 (75 %)	
3	206 (23 %)	201 (22 %)	
4	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	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 NTproBNP (pg/ml)	1423 [822 , 2756]	1457 [831 , 2816]	0.62
hs-troponin T (ng/L)	16 [10, 25]	16 [10, 24]	0.54

Abbreviations: ICD = implantable cardiac defibrillator, CRT = cardiac resynchronization therapy, LV = left ventricular, HF = heart failure, ACEi = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker, BNP = b-type natriuretic peptide, NTproBNP = n-terminal pro BNP, NYHA = New York heart association

Table 2: Baseline Biomarker Data

Marker	PARADIGM-HF Median (IQR)	Referent Controls Median (IQR)	%Pts 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%

Abbreviations: TIMP = tissue inhibitor of matrix metalloproteinase (MMP), PINP = n-terminal propeptide of collagen I and collagen III (PIIINP), IQR = interquartile range, ↑, ↓ represent the directional differences between PARADIGM –HF patients and mean referent control values and the numerical value of that change.

Table 3: Relationships Between Biomarkers and Outcomes

Marker	Visit	Median [IQ Range]	Baseline levels vs outcomes HR (95% CI), p-value Log-transformed, per SD		8-mo changes vs subsequent outcomes HR (95% CI), p-value, per 20% increase	
			Primary outcome*	CV Death*	Primary outcome**	CV Death**
Aldo (pmol/L)	Baseline	275 [173,464]	0.96 (0.87-1.07) p=0.47	1.00 (0.87-1.16) p=0.97	0.97 (0.93-1.01) p=0.16	0.97 (0.92-1.02) p=0.27
	M8	243 [154,394]				
sST2 (ng/mL)	Baseline	32.2 [25.4,41.5]	1.16 (1.06-1.28) p=0.002	1.18 (1.03-1.35) p=0.014	1.14 (1.06-1.23) p<0.001	1.05 (0.96-1.16) p=0.28
	M8	31.0 [24.7,39.3]				
TIMP-1 (ng/mL)	Baseline	125 [106,152]	1.21 (1.07-1.37) p=0.003	1.55 (1.28-1.87) p<0.001	1.03 (0.94-1.13) p=0.50	1.19 (1.05-1.35) p=0.006
	M8	123 [102,152]				
MMP-2 (ng/mL)	Baseline	135 [117,158]	1.07 (0.96-1.20) p=0.24	1.06 (0.90-1.26) p=0.49	1.03 (0.94-1.12) p=0.54	1.03 (0.92-1.15) p=0.62
	M8	133 [114,153]				
MMP-9 (ng/mL)	Baseline	64.1 [38.2,126.2]	1.02 (0.92-1.14) p=0.66	0.99 (0.85-1.16) p=0.94	1.00 (0.97-1.03) p=0.93	0.99 (0.95-1.04) p=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) p=0.35	1.04 (0.91-1.20) p=0.54	1.09 (1.00-1.20) p=0.06	1.08 (0.96-1.22) p=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) p=0.76	1.04 (0.87-1.24) p=0.67	1.01 (0.95-1.08) p=0.67	1.03 (0.95-1.13) p=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) p=0.11	1.24 (1.03-1.49) p=0.025	1.05 (0.97-1.13) p=0.22	1.04 (0.95-1.14) p=0.39
	M8	4.5 [3.6, 5.8]				

*adjusted for treatment + baseline covariates (including log-transformed BNP, NT-proBNP, hsTnT)
**adjusted for treatment + baseline covariates (including log-transformed BNP, NT-proBNP, hsTnT) + baseline biomarker value

There were no statistically significant treatment interactions (all p>0.05)

Abbreviations: Baseline covariates = age, sex, geographic region, body mass index, New York Heart Association 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(hs-TnT); and randomized treatment (enalapril or sacubitril/valsartan). TIMP = tissue inhibitor of matrix metalloproteinase (MMP), PINP = n-terminal propeptide of collagen I and collagen III (PIIINP),

Appendix Table 1: Biomarker Standards

Biomarker (unit)	Manufacturer	COV%	LLOQ (or LOD)	Reporting Range	Matrix
Aldosterone (pmol/L)	DiaSorin (Liason)	< 6%	52.90	52.9 – 1664.6	
TIMP-1 (ng/mL)	R& D Systems	< 7%	0.1	0.1 - 8000	EDTA plasma
MMP-2 (pg/mL)	Meso Scale Discovery	< 15%	976	976 - 500,000	Li-Hep plasma
MMP-9 (pg/mL)	Meso Scale Discovery	< 15%	488	488 - 1,000,000	Li-Hep plasma
Galectin-3 (ng/mL)	BG Medicine	< 11.5%	1.74	1.74 – 96.6	serum
sST-2 (ng/mL)	Critical Diagnostics	< 2.5%	3.13 ng/mL	< 3.13 – 1600	serum
PINP (ug/L)	Orion RIA	≤ 10%	5 (LOD)	5 – 250	serum
PIIINP (ug/L)	Orion RIA	≤ 10%	1 (LOD)	1 – 50	serum
NT-proBNP (pg/mL)	Roche	< 2.5%	8	< 8 - 35,000	Li-Hep plasma
hsTnT (ng/mL)	Roche (5th generation)	14.5% < 10%	5 (LOD) 13 (LLOQ)	< 5 – 10,000	EDTA plasma

Abbreviations: TIMP = tissue inhibitor of matrix metalloproteinase (MMP), PINP = n-terminal propeptide of collagen I and collagen III (PIIINP), hsTnT = high sensitivity troponin, CV = coefficient of variance, LOD = lower limit of detection (LOD), LLOQ = lower limit of quantification, EDTA = Ethylenediaminetetraacetic acid, Li Hep = lithium heparin.

Appendix Table 2:

Biomarker	Referent Control	Reference	Assay Method Antibody
Aldosterone	7-30 ng/dL (or 70-300 pg/mL)	22	Textbook on WebMD
	5.31 ± 3.8 ng/dL (mean±sd) (n=2157) (or 53±38 pg/mL)	23	Vitros analyzer, Ortho Clinical Diagnosis
	7-20 ng/dL (70-200 ng/mL) or 0.2-0.8 nmol/L (200-800 pmol/L)	24	ACP Lab Reference Ranges
sST-2	20 (17, 26) ng/mL (median/iqr)	8	Presage immunoassay, Critical Diagnostics, San Diego
Galectin-3	12 (9, 15) ng/mL (median/iqr)	8	Enzyme-linked immunosorbent assay, BG Medicine, Waltham, MA
MMP-2	339.7 ± 9.3 ng/mL (mean±sem) (n=241)	7	Multiplex suspension array, MMP Base kit LMP000, BioPlex 200, BioRad Laboratories
	374.8 ± 35.7 ng/mL (mean±sem) (n=15)	14	Multiplex suspension array, MMP Base kit LMP000, BioPlex 200, BioRad Laboratories
	236.6 (217.6,275.3) ug/L (median/iqr) (n=49)	18	R&D Systems
	335 (323, 443) ng/mL (median/iqr)	8	Immunoassay (R&D Systems, Minneapolis, MN)
MMP-9	95.0 ± 3.8 ng/mL (mean±sem) (n=241)	7	Multiplex suspension array, MMP Base kit LMP000, BioPlex 200, BioRad Laboratories
	121.1 ± 13.1 ng/mL (mean±sem) (n=15)	14	Multiplex suspension array, MMP Base kit LMP000, BioPlex 200, BioRad Laboratories
	58.8 (38.0,134.4) ug/L (median/iqr) (n=49)	18	R&D Systems
	145 ± 88 ug/L (mean±sd) (n=9)	19	ELISA, R&D Systems
TIMP-1	72.2 ± 1.4 ng/mL (mean±sem) (n=241)	7	Multiplex suspension array, TIMP MSA kit LKT003, BioPlex 200, BioRad Laboratories
	106.3 ± 4.4 ng/mL (mean±sem) (n=15)	14	Multiplex suspension array, TIMP MSA kit LKT003, BioPlex 200, BioRad Laboratories
	827.7 ± 111.5 ug/L (mean±sd) (n=92)	15	ELISA (Amersham Pharmacia Biotech, UK)
	217.2 (203.3,245.2) ug/L (median/iqr) (n=49)	18	R&D Systems
	165 ± 67 ug/L (mean±sd) (n=9)	19	ELISA, R&D Systems
	634 ± 7 ng/mL (mean±sem)	20	GE Healthcare Life Sciences
PINP	37.1 ± 1.3 ng/mL (mean±sem) (n=241)	7	Radioimmunoassay
	46.6 ± 19.1 ug/L (mean±sd) (n=92)	15	Radioimmunoassay, Orion Diagnostica
	53.4 ± 25.9 ug/L (mean±sd) (n=25)	17	Radioimmunoassay, Abbott
	39.6 ± 17.4 ug/L (mean±sd) (n=9)	19	Radioimmunoassay, Abbott

PIIINP	7.2 ± 0.1 ng/mL (mean±sem) (n=241)	7	Radioimmunoassay
	3.5 ± 1.3 ug/L (mean±sd) (n=92)	15	Radioimmunoassay, Orion Diagnostica
	3.1 (2.4,4.0) ng/mL (median/iqr) (n=283)	16	Radioimmunoassay, Orion Diagnostica
	4.4 ± 1.1 ug/L (mean±sd) (n=25)	17	Radioimmunoassay, Abbott
	5.8 (4.4 ,7.0) ug/L (median/iqr) (n=49)	18	Radioimmunoassay, Orion Diagnostica
	6.5 (6.1, 8.2) ng/mL (median/iqr)	8	Quest Diagnostics, Valencia, CA
	4.1 ± 0.7 ug/L (mean±sd) (n=9)	19	Radioimmunoassay, Abbott
	457 (175-1160) pg/mL (median/iqr)	20	MyBioSource, San Diego
	3.4 ± 0.2 ug/L (mean±sem) (n=30)	21	Radioimmunoassay, Farmos Diagnostica

Abbreviations: TIMP = tissue inhibitor of matrix metalloproteinase (MMP), PINP = n-terminal propeptide of collagen I and collagen III (PIIINP),

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 (7). 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 (25). Median (IQR) referent control data for sST-2 were aggregated from previously published studies (including the Framingham study) in which subjects of age, sex, and race distribution similar to this study population were examined (26-29). Reported normal values for sST-2 were 20 ng/mL (95% CI of 17-26 ng/mL), and for Gal-3 was 12 ng/mL (95% CI of 9-15 ng/mL) (8). 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 have similar sex distribution, the referent control values listed in Appendix Table 2 represent the total referent population examined.

Appendix Table 3: Relationship Between Profibrotic Biomarker and Outcome: Independent effect of each Biomarker

Marker	Baseline levels vs outcomes HR (95% CI), p-value log transformed per SD	
	Primary outcome*	**CV Death***
Aldo (pmol/L)	0.89 (0.78-1.01) p=0.07	0.91 (0.76-1.09) p=0.30
sST2 (ng/mL)	1.11 (0.98-1.25) p=0.09	1.08 (0.91-1.29) p=0.37
TIMP-1 (ng/mL)	1.20 (1.03-1.38) p=0.017	1.43 (1.14-1.79) p=0.002
MMP-2 (ng/mL)	1.02 (0.90-1.16) p=0.74	1.00 (0.83-1.20) p=0.99
MMP-9 (ng/mL)	1.02 (0.91-1.14) p=0.74	1.01 (0.85-1.20) p=0.93
Galectin-3 (ng/mL)	1.08 (0.95-1.23) p=0.26	1.04 (0.86-1.26) p=0.67
PINP (ng/mL)	0.90 (0.77-1.04) p=0.16	0.89 (0.72-1.11) p=0.32
PIIINP (ng/mL)	1.07 (0.91-1.25) p=0.44	1.14 (0.90-1.44) p=0.29

Abbreviations: *adjusted for treatment + baseline covariates (including BNP, NT-proBNP, hsTnT) + all 8 profibrotic biomarkers,

TIMP = tissue inhibitor of matrix metalloproteinase (MMP), PINP = n-terminal propeptide of collagen I and collagen III (PIIINP),

Appendix Table 4: Compare c-statistic to determine if fibrosis biomarkers added independent value to prognosis.

(treatment included in all)	**Primary Outcome**	**CV Death**
M1: Baseline Covariates	0.66	0.67
M2: M1 + BNP, NT-proBNP, hsTnT	0.71	0.70
M3: M2 + TIMP-1	0.72 (p=0.08 vs M2)	0.71 (p=0.37 vs M2)
M4: M2 + all 8 profibrotic biomakers	0.72	0.72
M5: BNP, NT-proBNP, hsTnT + all 8 profibrotic biomarkers (i.e. M4 minus M1)	0.70	0.68

Abbreviations: BNP = b-type natriuretic peptide, NTproBNP = n-terminal pro BNP, TIMP = tissue inhibitor of matrix metalloproteinase (MMP), PINP = n-terminal propeptide of collagen I and collagen III (PIIINP),

Appendix Table 5: Baseline Demographics

Patients in Paradigm-HF with biomarker vs no biomarker data	**No Baseline Biomarkers**	**Baseline Biomarkers**	
	n=6332	**n=2067**	**p-value**
Age (years)	63 ± 12	67 ± 10	<0.001
Female sex	1446 (23 %)	386 (19 %)	<0.001
Body Mass Index	27.7 ± 5.5	29.5 ± 5.4	<0.001
NYHA Class			<0.001
1	342 (5 %)	47 (2 %)	
2	4410 (70 %)	1509 (73 %)	
3	1520 (24 %)	498 (24 %)	
4	49 (1 %)	11 (1 %)	
LV Ejection Fraction	29 ± 6	30 ± 6	<0.001
Prior use of ACEi	4865 (77 %)	1667 (81 %)	<0.001
Prior use of ARB	1478 (23 %)	414 (20 %)	0.002
Prior HF hospitalization	4035 (64 %)	1239 (60 %)	0.001
Hypertension status	4333 (68 %)	1607 (78 %)	<0.001
Race			<0.001
White	3582 (57 %)	1962 (95 %)	
Black	364 (6 %)	64 (3 %)	
Asian	1501 (24 %)	8 (0 %)	

Other	885 (14 %)	33 (2 %)	
Region			<0.001
North America	265 (4 %)	337 (16 %)	
Latin America	1433 (23 %)	0 (0 %)	
Western Europe and Other	1112 (18 %)	939 (45 %)	
Central/Eastern Europe	2035 (32 %)	791 (38 %)	
Asia-Pacific	1487 (23 %)	0 (0 %)	
Systolic Blood Pressure	121 ± 15	123 ± 16	<0.001
Diabetes Mellitus	2089 (33 %)	818 (40 %)	<0.001
Heart Rate	73 ± 12	71 ± 12	<0.001
Ischemic Cardiomyopathy	3711 (59 %)	1325 (64 %)	<0.001
Prior Myocardial Infarction	2628 (42 %)	1006 (49 %)	<0.001
Prior Atrial Fibrillation	2091 (33 %)	1000 (48 %)	<0.001
Prior Stroke	515 (8 %)	210 (10 %)	0.004
ICD	656 (10 %)	587 (28 %)	<0.001
CRT	345 (5 %)	229 (11 %)	<0.001
Diuretic	5045 (80 %)	1693 (82 %)	0.027
Beta Blockers	5841 (92 %)	1970 (95 %)	<0.001
Digoxin	2074 (33 %)	465 (22 %)	<0.001
Aldosterone	3740 (59 %)	931 (45 %)	<0.001
Baseline Creatinine	1.1 ± 0.3	1.2 ± 0.3	<0.001

Baseline BNP (pg/ml)	261 [154 , 496]	229 [154 , 400]	<0.001
Baseline NTproBNP (pg/ml)	1665 [903 , 3385]	1485 [852 , 2907]	<0.001
hs-troponin T (ng/L)	24 [17, 31]	16 [11, 25]	0.40

Abbreviations: ICD = implantable cardiac defibrillator, CRT = cardiac resynchronization therapy, LV = left ventricular, HF = heart failure, ACEi = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker, BNP = b-type natriuretic peptide, NTproBNP = n-terminal pro BNP, NYHA = New York heart association

Appendix Table 6: **Baseline Demographics**

Patients from full Paradigm-HF cohort vs those with baseline biomarkers vs those with both baseline and follow-up biomarkers	**PARADIGM-HF cohort**	**Baseline Biomarkers**	**Full cohort vs Baseline biomarker cohort**	**Baseline + follow-up biomarkers**	**Baseline only vs Baseline + follow-up**
	n=8399	**n=2067**	**p-value**	**n=1776**	**p-value**
Age (years)	64 ± 11	67 ± 10	<0.001	67 ± 10	0.027
Female sex	1832 (22 %)	386 (19 %)	<0.001	332 (19 %)	0.96
Body Mass Index	28.2 ± 5.5	29.5 ± 5.4	<0.001	29.6 ± 5.5	0.005
NYHA Class			<0.001		0.021
1	389 (5 %)	47 (2 %)		40 (2 %)	
2	5919 (71 %)	1509 (73 %)		1317 (74 %)	
3	2018 (24 %)	498 (24 %)		407 (23 %)	
4	60 (1 %)	11 (1 %)		10 (1 %)	
LV Ejection Fraction	29 ± 6	30 ± 6	<0.001	31 ± 6	0.001
Prior use of ACEi	6532 (78 %)	1667 (81 %)	<0.001	1427 (80 %)	0.39
Prior use of ARB	1892 (23 %)	414 (20 %)	0.002	363 (20 %)	0.25
Prior HF hospitalization	5274 (63 %)	1239 (60 %)	0.001	1047 (59 %)	0.023
Hypertension status	5940 (71 %)	1607 (78 %)	<0.001	1372 (77 %)	0.18
Race			<0.001		<0.001
White	5544 (66 %)	1962 (95 %)		1700 (96 %)	
Black	428 (5 %)	64 (3 %)		44 (2 %)	
Asian	1509 (18 %)	8 (0 %)		6 (0 %)	
Other	918 (11 %)	33 (2 %)		26 (1 %)	

Region			<0.001		0.016
North America	602 (7 %)	337 (16 %)		276 (16 %)	
Latin America	1433 (17 %)	0 (0 %)		0 (0 %)	
Western Europe and Other	2051 (24 %)	939 (45 %)		802 (45 %)	
Central Europe	2826 (34 %)	791 (38 %)		698 (39 %)	
Asia-Pacific	1487 (18 %)	0 (0 %)		0 (0 %)	
Systolic Blood Pressure	121 ± 15	123 ± 16	<0.001	123 ± 16	0.21
Diabetes Mellitus	2907 (35 %)	818 (40 %)	<0.001	705 (40 %)	0.78
Heart Rate	72 ± 12	71 ± 12	<0.001	71 ± 12	0.08
Ischaemic Cardiomyopathy	5036 (60 %)	1325 (64 %)	<0.001	1139 (64 %)	0.94
Prior Myocardial Infarction	3634 (43 %)	1006 (49 %)	<0.001	866 (49 %)	0.84
Prior Atrial Fibrillation	3091 (37 %)	1000 (48 %)	<0.001	864 (49 %)	0.54
Prior Stroke	725 (9 %)	210 (10 %)	0.004	178 (10 %)	0.61
ICD	1243 (15 %)	587 (28 %)	<0.001	498 (28 %)	0.37
CRT	574 (7 %)	229 (11 %)	<0.001	190 (11 %)	0.17
Diuretic	6738 (80 %)	1693 (82 %)	0.027	1444 (81 %)	0.08
Beta Blockers	7811 (93 %)	1970 (95 %)	<0.001	1695 (95 %)	0.48
Digoxin	2539 (30 %)	465 (22 %)	<0.001	394 (22 %)	0.40
Aldosterone	4671 (56 %)	931 (45 %)	<0.001	798 (45 %)	0.81
Baseline Creatinine	1.1 ± 0.3	1.2 ± 0.3	<0.001	1.2 ± 0.3	<0.001
Baseline BNP (pg/ml)	253 [154 , 468]	229 [154 , 400]	<0.001	221 [149 , 384]	<0.001
Baseline NTproBNP (pg/ml)	1612 [886 , 3224]	1485 [852 , 2907]	<0.001	1444 [827 , 2788]	<0.001
hs-troponin T (ng/L)	17 [11, 25]	16 [11, 25]	0.40	16 [10, 24]	<0.001

Abbreviations: ICD = implantable cardiac defibrillator, CRT = cardiac resynchronization therapy, LV = left ventricular, HF = heart failure, ACEi = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker, BNP = b-type natriuretic peptide, NTproBNP = n-terminal pro BNP, NYHA = New York heart association

Appendix Table 7: Effects of sacubitril/valsartan on profibrotic biomarkers adjusted for systolic blood pressure

Marker	Visit	Baseline to 8 Mo change:					
				*Adjusted for baseline biomarker		** Additionally adjusted for baseline SBP + SBP change	
		Enalapril	Sacubitril/ valsartan	Sacubitril/ valsartan effect*	P-value*	Sacubitril/ valsartan effect**	P-value**
Aldo (pmol/L)	IQR	[-34%, +26%]	[-37%, +21%]		--		
	% change	-10.3%	-14.2%	-6% (-11%, -1%)	0.020	-7% (-12%, -2%)	0.007
sST2 (ng/ml)	IQR	[-14%, +18%]	[-18%, +10%]		--		--
	% change	+0.8%	-5.5%	-7% (-9%, -4%)	<0.001	-6% (-9%, -4%)	<0.001
TIMP-1 (ng/ml)	IQR	[-11%, +15%]	[-15%, +10%]		--		--
	% change	+0.3%	-3.6%	-4% (-7%, -1%)	0.003	-5% (-7%, -2%)	0.001
MMP-2	IQR	[-10%, +7.6%]	[-11%, +7.4%]				
	% change	-0.8%	-2.1%	-1% (-4%, +1%)	0.36	-1% (-4%, +2%)	0.46
MMP-9	IQR	[-37%, +54%]	[-43%, +47%]				
	% change	-3.0%	-10.5%	-8% (-14%, -2%)	0.010	-9% (-15%, -3%)	0.006
Gal-3	IQR	[-7.4%, +23%]	[-8.6%, +21%]				
	% change	+5.3%	+4.7%	-1% (-3%, +2%)	0.51	-1% (-4%, +1%)	0.24
PINP	IQR	[-20%, +26%]	[-26%, +18%]				
	% change	+0.6%	-6.2%	-6% (-10%, -3%)	<0.001	-6% (-10%, -3%)	<0.001
PIIINP	IQR	[-22%, +22%]	[-22%, +18%]				
	% change	-1.7%	-4.5%	-3% (-6%, 0%)	0.086	-3% (-6%, 0%)	0.090

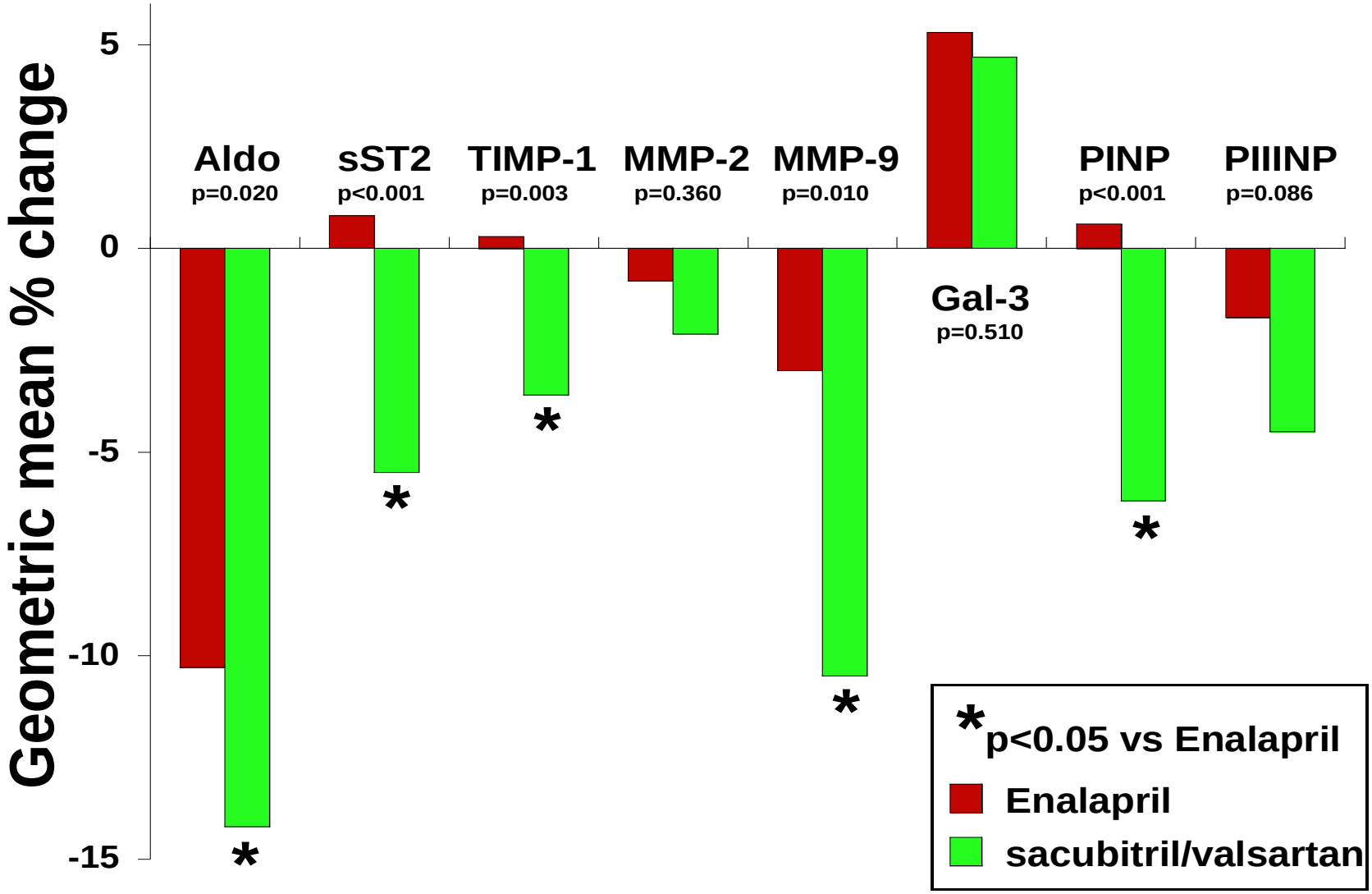

Geometric mean % change
5
0
-5
-10
-15
Aldo
p=0.020
sST2
p<0.001
TIMP-1
p=0.003
MMP-2
p=0.360
MMP-9
p=0.010
Gal-3
p=0.510
PINP
p<0.001
PIIINP
p=0.086
*
*
*
*
*
*p<0.05 vs Enalapril
Enalapril
sacubitril/valsartan

Figure 1.

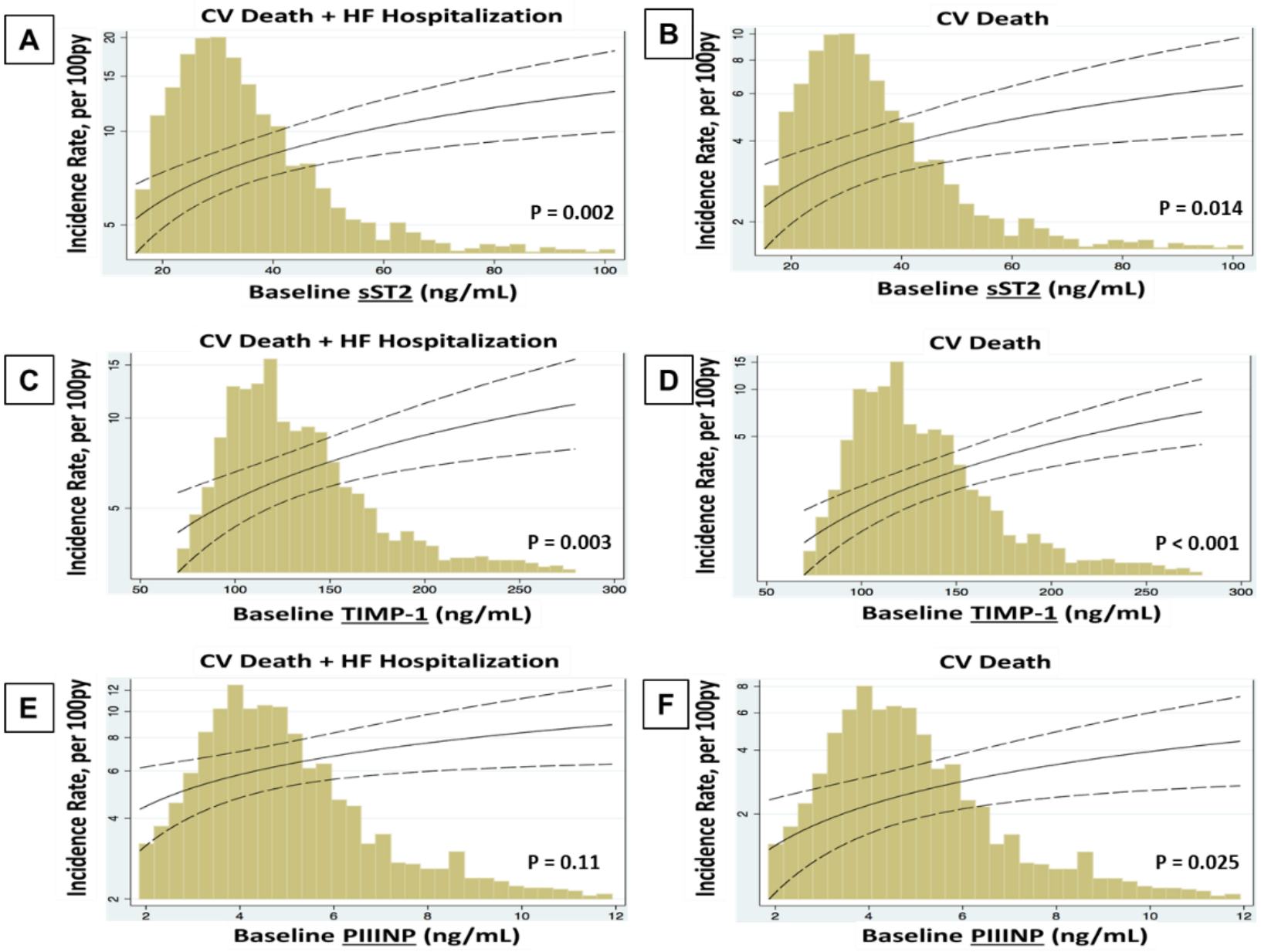

A
CV Death + HF Hospitalization
Incidence Rate, per 100py
P = 0.002
Baseline sST2 (ng/mL)
B
CV Death
Incidence Rate, per 100py
P = 0.014
Baseline sST2 (ng/mL)
C
CV Death + HF Hospitalization
Incidence Rate, per 100py
P = 0.003
Baseline TIMP-1 (ng/mL)
D
CV Death
Incidence Rate, per 100py
P < 0.001
Baseline TIMP-1 (ng/mL)
E
CV Death + HF Hospitalization
Incidence Rate, per 100py
P = 0.11
Baseline PIIINP (ng/mL)
F
CV Death
Incidence Rate, per 100py
P = 0.025
Baseline PIIINP (ng/mL)

Figure 2.

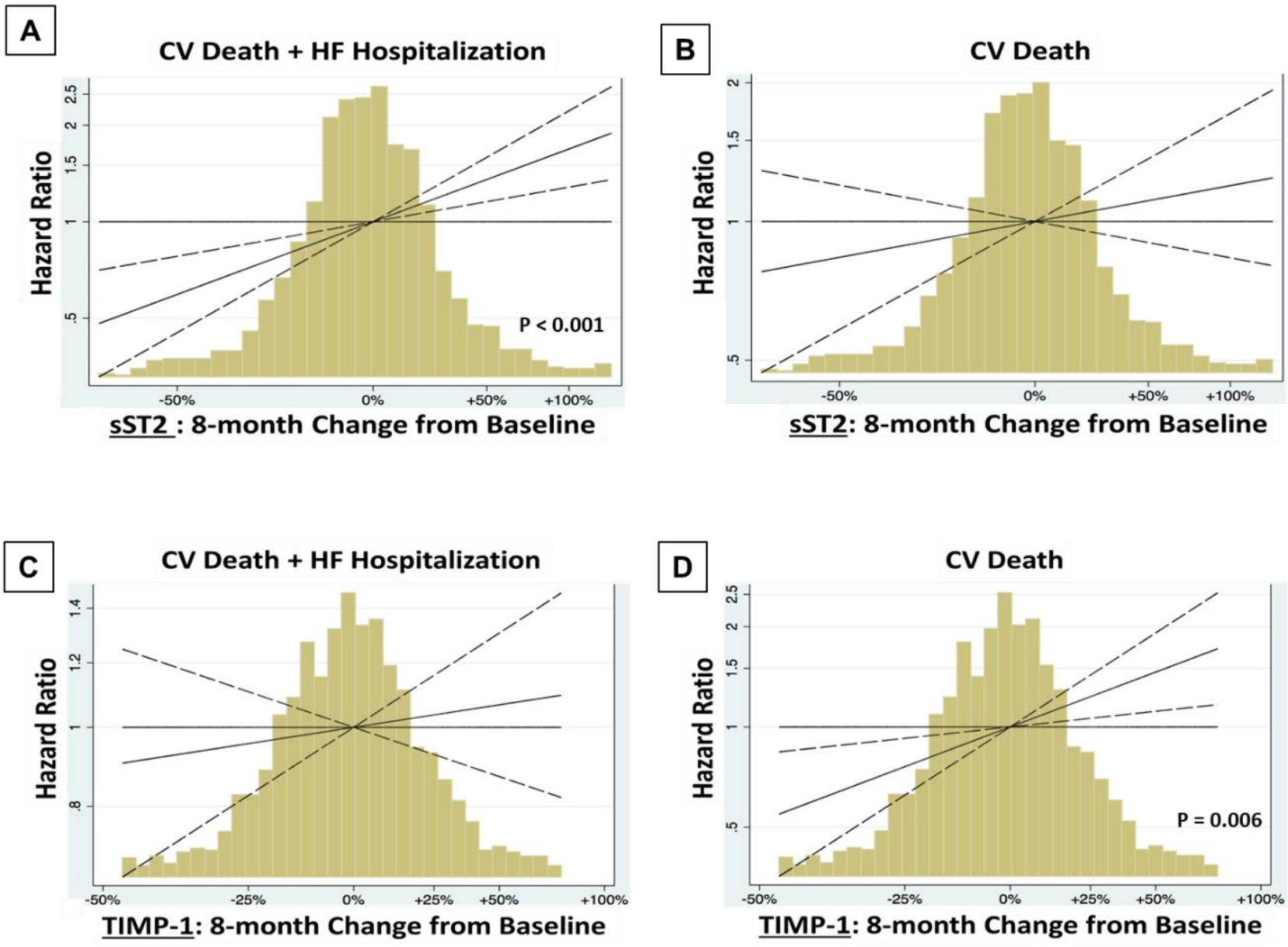
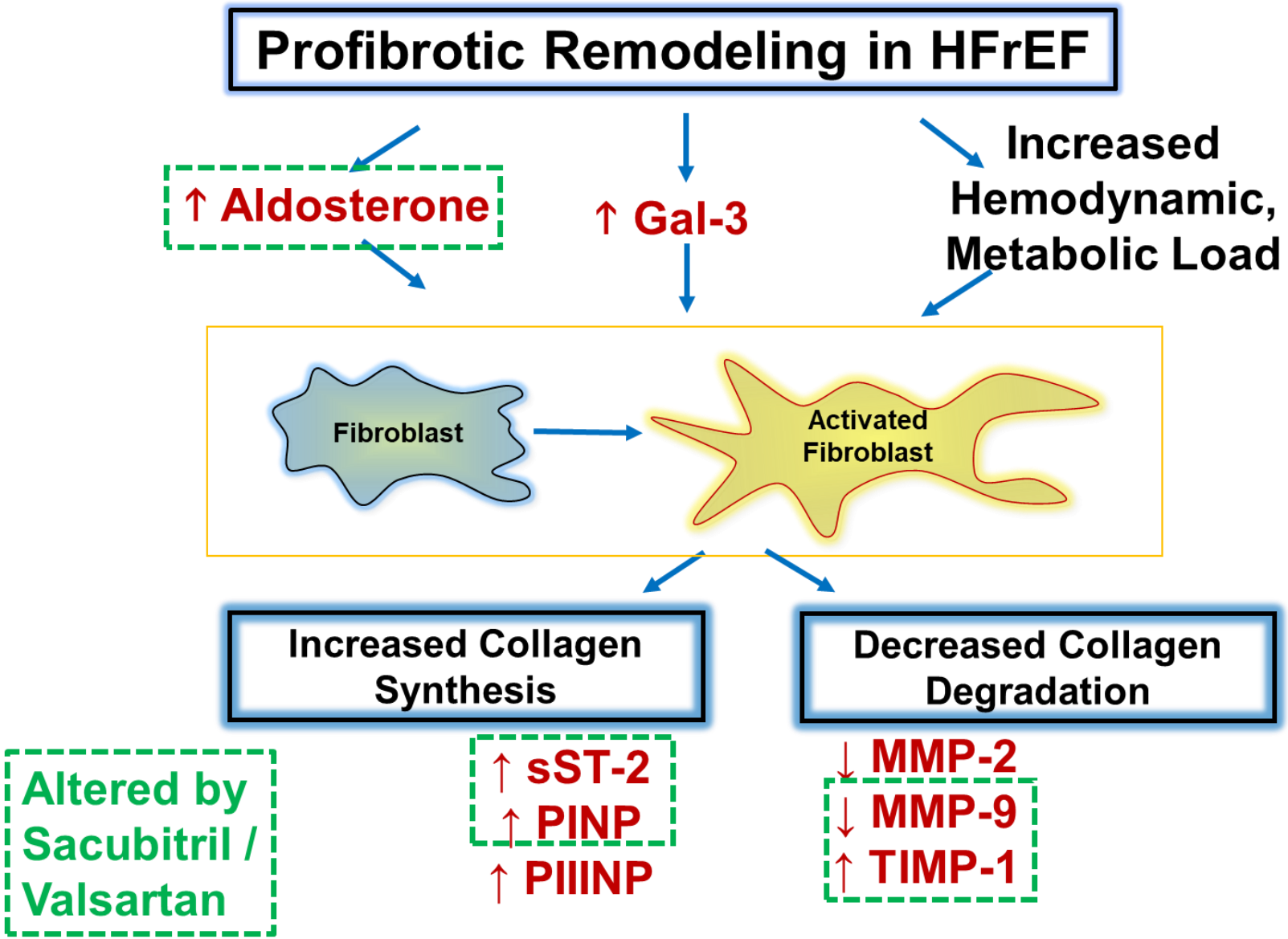

A
CV Death + HF Hospitalization
Hazard Ratio
P < 0.001
-50%
0%
+50%
+100%
sST2 : 8-month Change from Baseline
B
CV Death
Hazard Ratio
-50%
0%
+50%
+100%
sST2: 8-month Change from Baseline
C
CV Death + HF Hospitalization
Hazard Ratio
-50%
-25%
0%
+25%
+50%
+100%
TIMP-1: 8-month Change from Baseline
D
CV Death
Hazard Ratio
P = 0.006
-50%
-25%
0%
+25%
+50%
+100%
TIMP-1: 8-month Change from Baseline

Figure 3.



Central Illustration Figure 4.

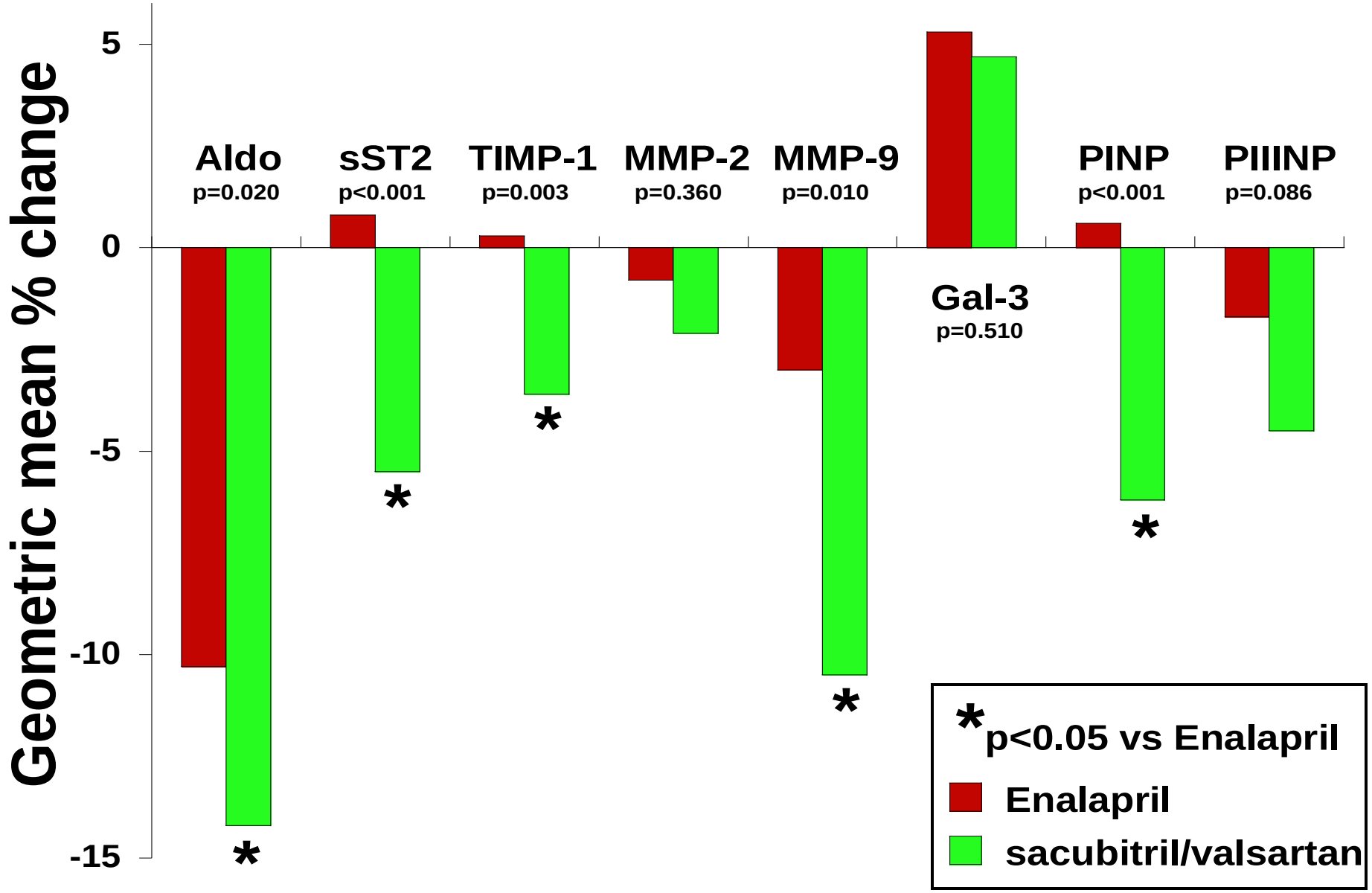

Geometric mean % change
5
0
-5
-10
-15
Aldo
p=0.020
sST2
p<0.001
TIMP-1
p=0.003
MMP-2
p=0.360
MMP-9
p=0.010
Gal-3
p=0.510
PINP
p<0.001
PIIINP
p=0.086
*
*
*
*
*
*p<0.05 vs Enalapril
Enalapril
sacubitril/valsartan

Figure 1

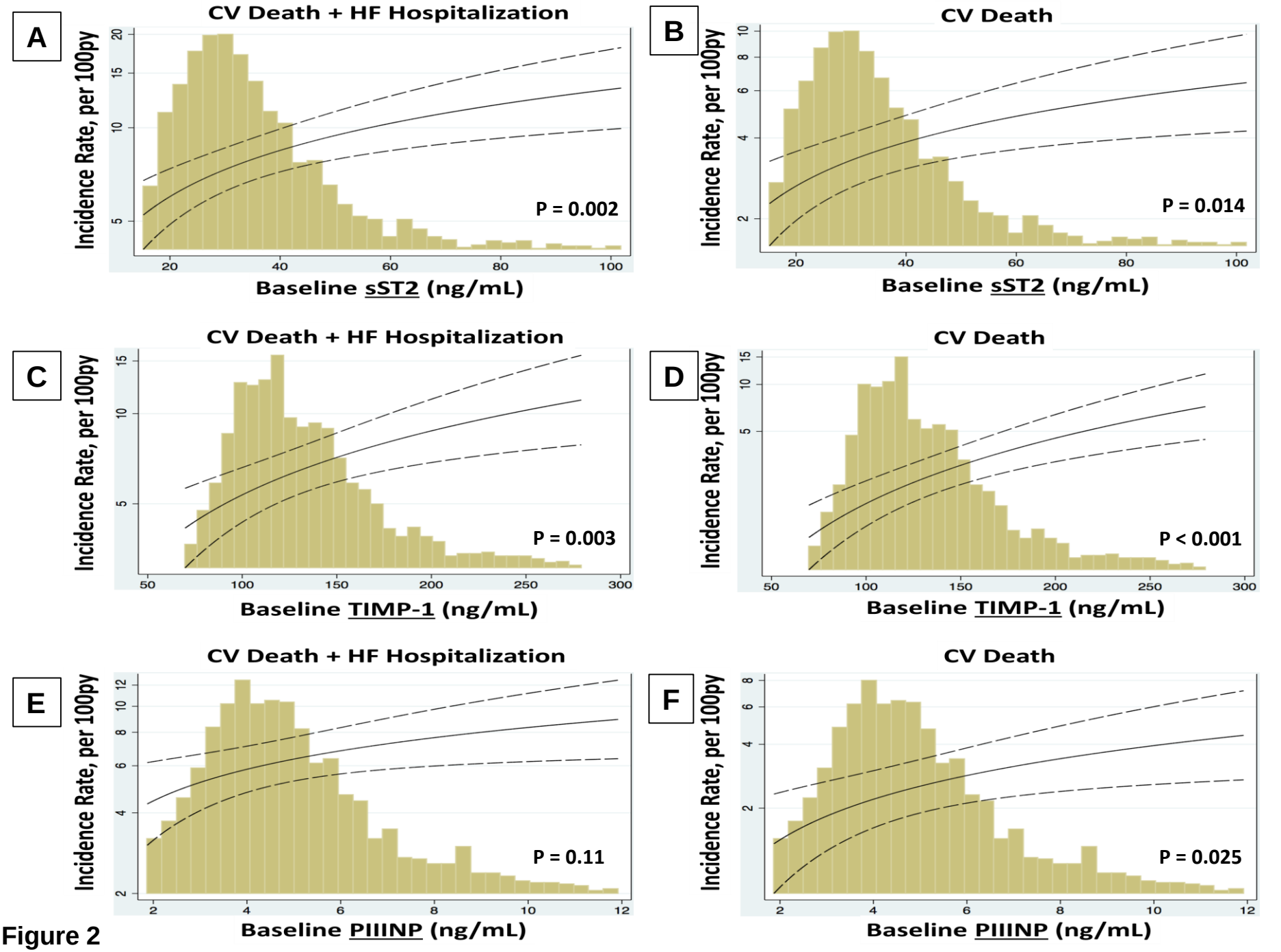

A
CV Death + HF Hospitalization
Incidence Rate, per 100py
P = 0.002
Baseline sST2 (ng/mL)
B
CV Death
Incidence Rate, per 100py
P = 0.014
Baseline sST2 (ng/mL)
C
CV Death + HF Hospitalization
Incidence Rate, per 100py
P = 0.003
Baseline TIMP-1 (ng/mL)
D
CV Death
Incidence Rate, per 100py
P < 0.001
Baseline TIMP-1 (ng/mL)
E
CV Death + HF Hospitalization
Incidence Rate, per 100py
P = 0.11
Baseline PIIINP (ng/mL)
F
CV Death
Incidence Rate, per 100py
P = 0.025
Baseline PIIINP (ng/mL)

Figure 2

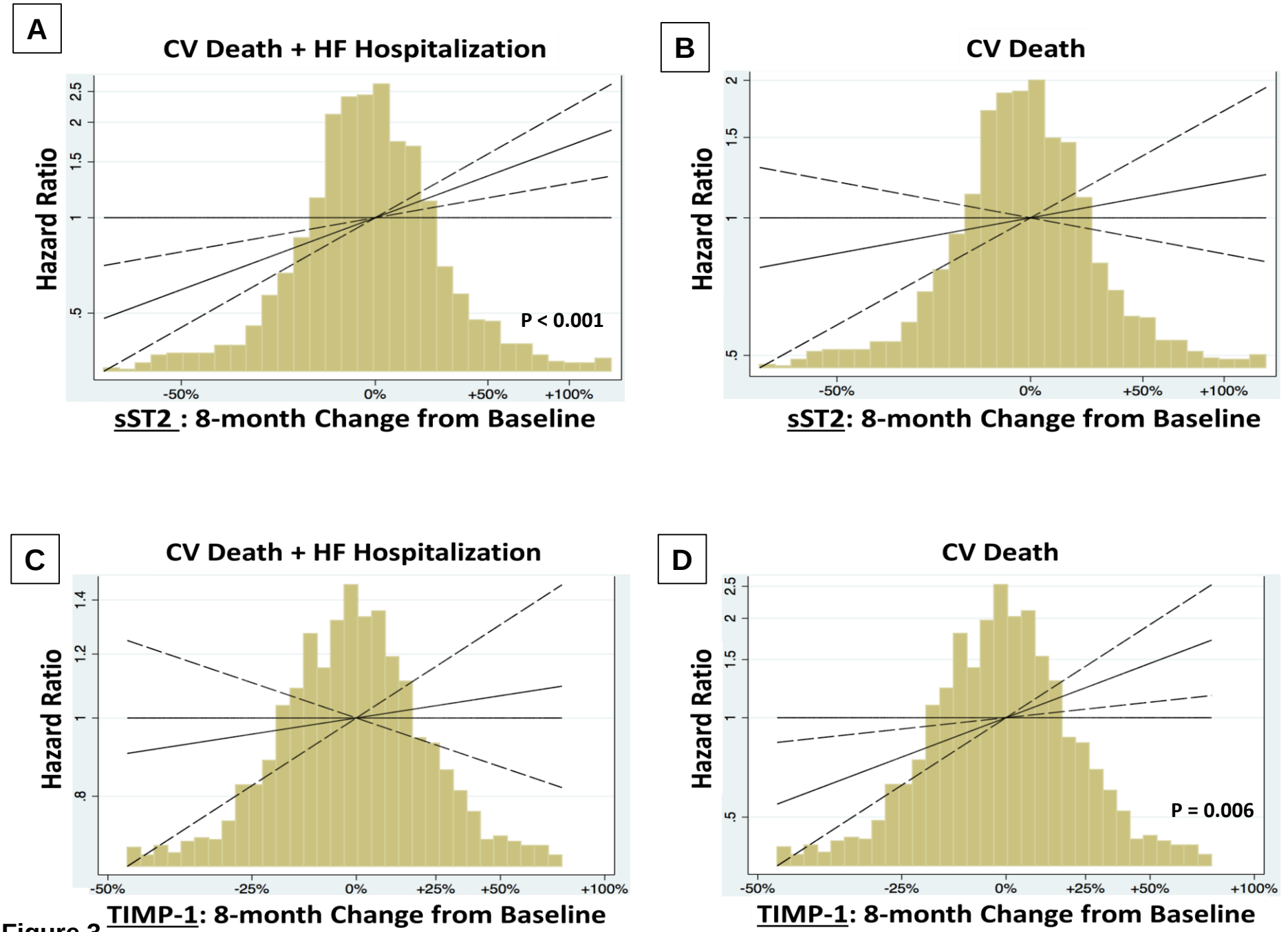

A
CV Death + HF Hospitalization
Hazard Ratio
.5
1
1.5
2
2.5
P < 0.001
-50%
0%
+50%
+100%
sST2 : 8-month Change from Baseline
B
CV Death
Hazard Ratio
.5
1
1.5
2
-50%
0%
+50%
+100%
sST2: 8-month Change from Baseline
C
CV Death + HF Hospitalization
Hazard Ratio
.8
1
1.2
1.4
-50%
-25%
0%
+25%
+50%
+100%
TIMP-1: 8-month Change from Baseline
D
CV Death
Hazard Ratio
.5
1
1.5
2
2.5
P = 0.006
-50%
-25%
0%
+25%
+50%
+100%
TIMP-1: 8-month Change from Baseline

Figure 3

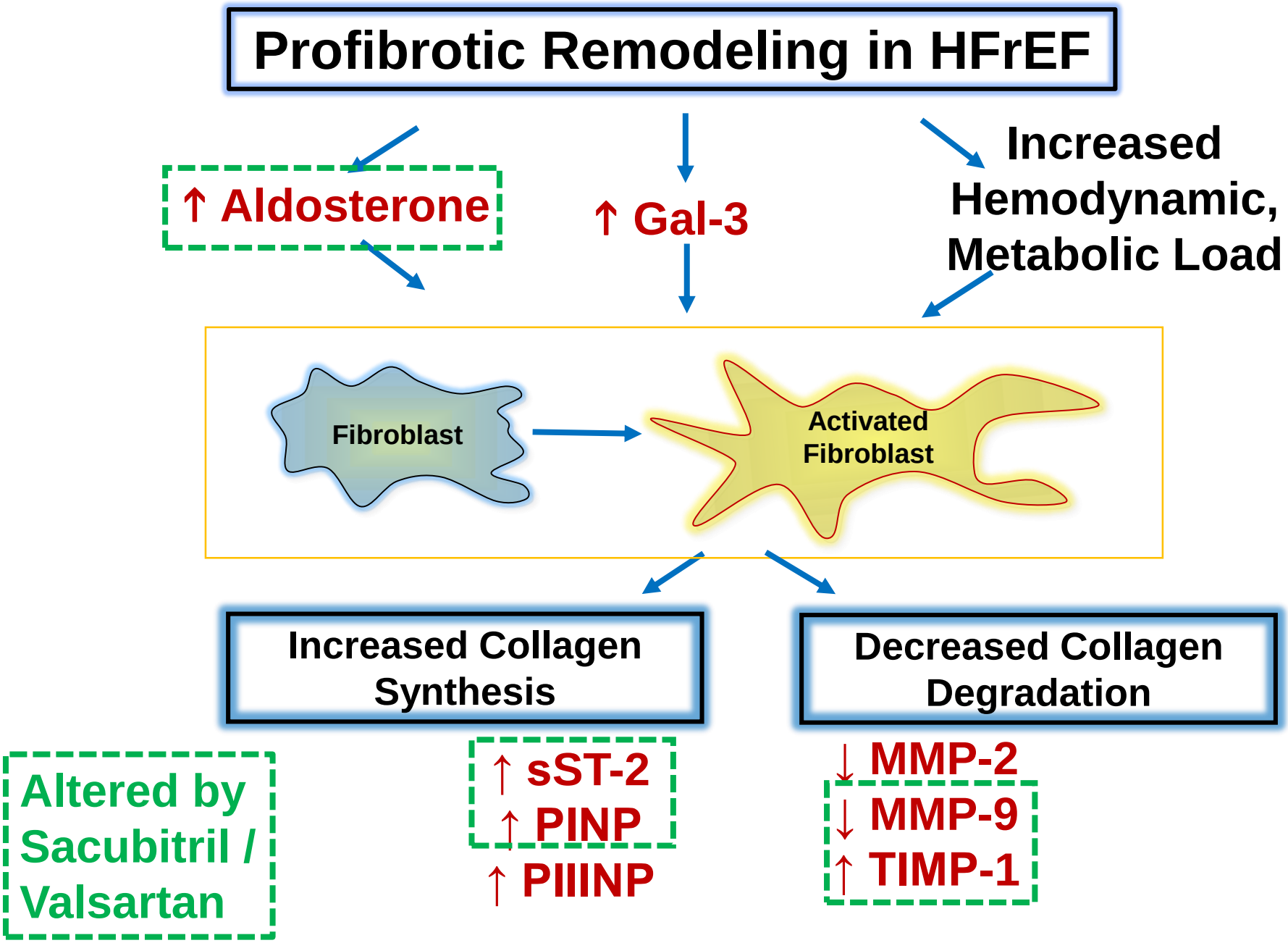


Figure 4 CENTRAL ILLUSTRATION