

Soluble ST2 in Heart Failure With Preserved Ejection Fraction

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Background—Soluble ST2 is a biomarker that is elevated in certain systemic inflammatory diseases. Comorbidity-driven microvascular inflammation is postulated to play a key role in heart failure with preserved ejection fraction (HFpEF) pathophysiology, but data on how sST2 relates to clinical characteristics or inflammatory conditions or biomarkers in HFpEF are limited. We sought to determine circulating levels and clinical correlates of sST2 in HFpEF.

Methods and Results—At enrollment, patients (n=174) from the Phosphodiesterase-5 Inhibition to Improve Clinical Status And Exercise Capacity in Diastolic Heart Failure (RELAX) trial of sildenafil in HFpEF had sST2 levels measured. Clinical characteristics; cardiac structure and function; exercise performance; and biomarkers of neurohumoral activation, systemic inflammation and fibrosis, and myocardial necrosis were assessed in relation to sST2 levels. Median sST2 levels in male and female HFpEF patients were 36.7 ng/mL (range 30.9–49.2 ng/mL; reference range 4–31 ng/mL) and 30.8 ng/mL (range 25.3–39.3 ng/mL; reference range 2–21 ng/mL), respectively. Among HFpEF patients, higher sST2 levels were associated with the presence of diabetes mellitus; atrial fibrillation; renal dysfunction; right ventricular pressure overload and dysfunction; systemic congestion; exercise intolerance; and biomarkers of systemic inflammation and fibrosis, neurohumoral activation, and myocardial necrosis ($P < 0.05$ for all). sST2 was not associated with left ventricular structure or left ventricular systolic or diastolic function.

Conclusions—In HFpEF, sST2 levels were associated with proinflammatory comorbidities, right ventricular pressure overload and dysfunction, and systemic congestion but not with left ventricular geometry or function. These data suggest that sST2 may be a marker of systemic inflammation in HFpEF and potentially of extracardiac origin.

Clinical Trial Registration—URL: <http://www.clinicaltrials.gov>. Unique identifier: NCT00763867. (*J Am Heart Assoc.* 2017;6:e004382. DOI: 10.1161/JAHA.116.004382.)

Key Words: biomarker • diastolic heart failure

ST2 is a member of the interleukin-1 family of receptors and exists as transmembrane (ST2L) and soluble (sST2) isoforms differentially regulated by alternative splicing.^{1,2}

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Accompanying Tables S1 through S3 are available at <http://jaha.ahajournals.org/content/6/2/e004382/DC1/embed/inline-supplementary-material-1.pdf>

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Received August 1, 2016; accepted January 4, 2017.

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Interleukin-33 (IL-33) binds to ST2L to elicit downstream signaling, whereas sST2 acts as a nonfunctional decoy IL-33 receptor, limiting IL-33/ST2L signaling.³ In patients with autoimmune inflammatory conditions, sST2 levels are elevated; in related experimental models, IL-33/ST2L signaling is proinflammatory and exacerbates clinical severity, whereas sST2 administration abrogates IL-33/ST2L signaling and is protective.^{3,4} In contrast, in cardiomyocytes and experimental heart failure (HF), IL-33/ST2L signaling is activated with cardiomyocyte/cardiac mechanical stress and neurohumoral activation but exerts protective cardiac antiremodeling effects. In these models, sST2 administration worsens the cardiac phenotype.^{4,5}

Although elevated sST2 levels have been repeatedly shown to be associated with worse outcomes in HF with reduced ejection fraction (HFrEF),^{6–16} sST2 appears to be of extracardiac origin in HFrEF.^{17–20} To date, sST2 has not been well studied in HF with preserved ejection fraction (HFpEF). In HFpEF, comorbidity-driven microvascular endothelial inflammation may play a key role in the genesis of myocardial,

vascular, skeletal muscle, and other end-organ damage,^{21–25} and IL-33/ST2 signaling appears to be involved in vascular endothelial cell inflammation and remodeling.^{17,26,27} Furthermore, once established, HF may itself perpetuate inflammation in proportion to the severity of systemic congestion and hypoperfusion.^{28,29} Thus, sST2 may be elevated in HFpEF caused by cardiomyocyte stress, comorbidity-driven systemic inflammation, or inflammation related to the severity of HF. Accordingly, in the comprehensively characterized HFpEF patients enrolled in the Phosphodiesterase-5 Inhibition to Improve Clinical Status And Exercise Capacity in Diastolic Heart Failure (RELAX) trial,³⁰ we determined whether circulating sST2 (henceforth ST2) levels were associated with the severity of cardiac remodeling and dysfunction, proinflammatory comorbidities, or markers of clinical HF severity.

Methods

The RELAX trial was conducted by the Heart Failure Clinical Research Network (HFN) and funded by the National Heart, Lung, and Blood Institute.³⁰ All patients provided written informed consent, and the trial was approved by the institutional review board at each participating site.

The design, entry criteria, and results of the RELAX trial were reported previously.^{30,31} Briefly, RELAX enrolled 216 outpatients who had ejection fraction $\geq 50\%$ and objective evidence of HF. In addition, patients were required to have elevated N-terminal pro-B-type natriuretic peptide (NT-proBNP; ≥ 400 pg/mL) or elevated invasively measured filling pressures and reduced exercise capacity ($\leq 60\%$ age, sex, and body size-specific predicted peak oxygen consumption). Patients with an estimated glomerular filtration rate (Modification of Diet in Renal Disease equation) < 20 mL/min per 1.73 m² were ineligible.

Participants underwent baseline studies that included a history and physical examination, echocardiography, cardiac magnetic resonance imaging if in sinus rhythm ($n=115$), cardiopulmonary exercise test, 6-minute walk test, Minnesota Living with Heart Failure Questionnaire, and phlebotomy for biomarker measurements.³¹

Comprehensive Doppler echocardiography and cardiac magnetic resonance imaging were performed according to study protocols³² with measurements performed at the HFN echocardiography (Mayo Clinic, Rochester, MN) and cardiac magnetic resonance imaging (Duke University, Durham, NC) core laboratories. The cardiopulmonary exercise test was performed according to a RELAX-specific protocol and interpreted by the HFN cardiopulmonary exercise test core laboratory (Massachusetts General Hospital, Boston, MA), as reported previously.³¹ Biomarker measurements were performed by the HFN biomarker core laboratory (University of Vermont, Burlington, VT), as described previously^{30,31} and included NT-proBNP, aldosterone, endothelin-1, cystatin-C, creatinine, uric acid, pro-

collagen III N-terminal peptide, C-telopeptide for type I collagen, high-sensitivity troponin I, and high-sensitivity C-reactive protein. ST2 levels were measured on banked samples by a high-sensitivity sandwich monoclonal immunoassay (Presage[®] ST2 assay; Critical Diagnostics). The normal values for ST2 are somewhat controversial, but in a carefully screened healthy Austrian cohort with normal inflammatory markers (C-reactive protein, procalcitonin, and interleukin-6) and normal BNP, sex-specific normal ranges were 4 to 31 ng/mL for male participants and 2 to 21 ng/mL for female participants using the Presage[®] ST2 assay.³³

Statistical Analysis

Data are presented as median (25th–75th percentiles) or number (percentage) across ST2 tertiles. Differences across ST2 tertiles were tested with Kruskal–Wallis, χ^2 , or Fisher exact tests, as appropriate. In healthy persons, ST2 levels are higher in men than in women but are not associated with age, body mass index, or renal function.^{33,34} Thus, multivariable least squares linear or logistic regression was used to assess the relationship between variables of interest and ST2 as a categorical variable (tertiles) adjusting for sex. With our sample size, we had 84% and 92% power to detect correlations of 0.22 and 0.25, respectively, between ST2 and other continuous measures. The associations between ST2 (log transformed) and other biomarkers (log transformed) are presented with Pearson correlation coefficients and were tested using linear regression models without and with sex adjustment. Analyses were performed by the HFN data coordinating center using SAS version 9.4 (SAS Institute). A $P < 0.05$ (2-sided) was considered statistically significant.

Results

Of the 216 participants enrolled in RELAX, stored serum from 174 patients was available to measure ST2 at enrollment. Included participants had a median age of 69 years, and 52% were men. Participants without stored serum availability were more likely to have had a HF hospitalization, worse renal function, and higher NT-proBNP levels (Table S1).

The median ST2 levels were 34.3 ng/mL (25th–75th percentiles 26.9–46.6 ng/mL) with a range from 14.4 to 197.9 ng/mL and higher levels in male participants (median 36.7 ng/mL [25th–75th percentiles 30.9–49.2 ng/mL]) than in female participants (median 30.8 ng/mL [25th–75th percentiles 25.3–39.3 ng/mL]) (Figure 1, Table 1). Accordingly, all results described in the following sections were adjusted for sex.

Clinical Characteristics and ST2 Levels in HFpEF

There was no association between ST2 levels and age, body size, or history of HF hospitalization (Table 1). However,

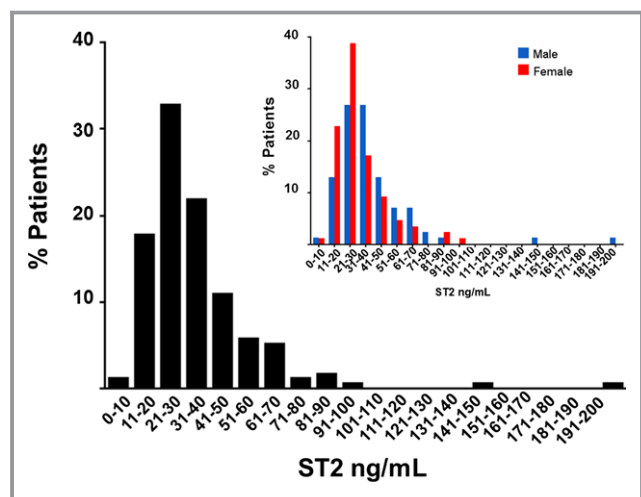


Figure 1. Frequency distribution of ST2 levels in heart failure with preserved ejection fraction overall and by sex (insert). The distribution of baseline ST2 levels in the RELAX trial cohort ($n=174$). Overall, median ST2 levels were 34.3 ng/mL (25th–75th percentiles 26.9–46.6 ng/mL) and were higher in male participants (36.7 ng/mL [25th–75th percentiles 30.9–49.2 ng/mL]) than in female participants (30.8 ng/mL [25th–75th percentiles 25.3–39.3 ng/mL]).

patients with higher ST2 levels were more likely to have diabetes mellitus ($P=0.005$), hypertension ($P=0.023$), atrial fibrillation or flutter ($P=0.049$), and renal dysfunction ($P<0.0001$) and were more likely to be treated with diuretics ($P=0.013$). Lung disease prevalence was similar across ST2 tertiles. Patients with higher ST2 levels also had more congestion with higher NT-proBNP levels ($P<0.0001$), a higher prevalence of jugular venous pressure elevation ($P=0.003$), more peripheral edema ($P=0.0006$), and worse New York Heart Association functional class ($P=0.029$), although ST2 levels were not associated with the Minnesota Living with Heart Failure Questionnaire score (Table 1).

Exercise Performance and ST2 Levels in HFpEF

Six-minute walk distance ($P=0.012$), peak oxygen consumption ($P=0.017$), percentage of predicted peak oxygen consumption ($P=0.017$), and peak systolic blood pressure ($P=0.005$) all declined with increasing ST2 levels (Table 2). There were nonsignificant trends toward lower peak heart rate ($P=0.07$) and chronotropic index ($P=0.08$) despite similar effort, as reflected in the respiratory exchange ratio. There was no association between ST2 levels and ventilatory efficiency (VE/VCO_2 slope).

Cardiac Structure and Function and ST2 Levels in HFpEF

There were no associations between ST2 levels and left ventricular (LV) diastolic or systolic function parameters or LV

geometry as assessed by echocardiography (Table 3) or in patients in sinus rhythm, by cardiac magnetic resonance (Table S2). Participants with higher ST2 levels had higher right ventricular (RV) systolic pressure ($P=0.016$) and worse RV function (lower tricuspid annular plane systolic excursion, $P=0.015$) by echocardiography (Table 3). In contrast, NT-proBNP levels were strongly associated with all diastolic function parameters, LV geometry, and RV load and systolic function but not with LV ejection fraction (Table S3).

ST2 and Other Biomarkers in HFpEF

ST2 concentrations were correlated with endothelin-1 ($r=0.33$, $P<0.0001$) and with biomarkers of systemic inflammation (high-sensitivity C-reactive protein, $r=0.22$, $P=0.002$), fibrosis (C-telopeptide for type I collagen, $r=0.30$, $P=0.0004$), and myocardial necrosis (high-sensitivity troponin-I, $r=0.33$, $P<0.0001$) but not with aldosterone or pro-collagen III N-terminal peptide (Figure 2).

Discussion

In this comprehensively phenotyped cohort of patients with HFpEF, ST2 levels were elevated compared with published normative sex-specific values. ST2 was higher in the presence of several proinflammatory comorbidities (diabetes mellitus, atrial fibrillation, renal dysfunction) and associated with greater RV pressure overload and dysfunction; central venous congestion; exercise intolerance; and biomarkers reflective of systemic inflammation and fibrosis, neurohumoral activation, and myocardial necrosis. ST2 levels were not associated with LV geometry or LV systolic or diastolic function. In contrast, NT-proBNP—a biomarker of myocardial origin, the production of which is stimulated by wall stress—correlated with the severity of LV remodeling and diastolic dysfunction. These data add to our understanding of ST2 in HFpEF and suggest that in HFpEF, ST2 is predominately a marker of systemic inflammation activated by interplay between proinflammatory comorbidities and HF severity-related systemic congestion.

Association of ST2 Levels With Sex

Consistent with findings in other cohorts,^{33–35} male RELAX participants had higher ST2 levels than their female counterparts. Although the mechanism of sex differences in ST2 levels remains unclear, the considerable differences of ST2 levels between sexes warrant adjustment for sex when analyzing the association between ST2 levels and disease severity.³⁶

ST2 as a Biomarker in HFpEF

Despite the lower percentage of male participants in RELAX compared with studies of ambulatory HFpEF patients, the

Table 1. Baseline Patient Characteristics by Tertiles of Baseline ST2 Levels

	Low ST2 Tertile (n=58)	Mid ST2 Tertile (n=58)	High ST2 Tertile (n=58)	P Value	P Value*
ST2 range, ng/mL	<29.5	29.5–38.5	>38.5
ST2, ng/mL	23.8 (21.7–26.9)	34.3 (31.6–36.5)	51.1 (46.6–66.4)
Male	18 (31)	32 (55)	36 (62)	0.002	...
Age, y	67 (61–74)	71 (64–79)	69 (63–77)	0.16	0.30
Body mass index, kg/m ²	32.9 (28.6–36.9)	32.5 (28.0–38.8)	33.8 (28.6–40.1)	0.54	0.70
Body surface area, m ²	2.13 (1.95–2.23)	2.07 (1.89–2.39)	2.12 (1.95–2.29)	0.80	0.53
HF hospitalization	18 (31)	16 (28)	21 (36)	0.60	0.63
Comorbidities					
Hypertension	44 (76)	56 (97)	46 (79)	0.005	0.023
Ischemic heart disease	20 (34)	20 (34)	27 (47)	0.30	0.50
Atrial fibrillation	21 (36)	30 (52)	34 (59)	0.047	0.049
COPD	8 (14)	11 (19)	13 (22)	0.48	0.77
Diabetes mellitus	17 (29)	19 (33)	35 (60)	0.001	0.005
Creatinine, mg/dL (n=172)	1.0 (0.8–1.3)	1.0 (0.9–1.2)	1.2 (0.9–1.7)	0.016	0.018
Cystatin-C, mg/L	1.15 (0.92–1.46)	1.21 (1.08–1.47)	1.58 (1.13–2.15)	0.0003	<0.0001
Medications					
ACEI or ARB	40 (69)	41 (71)	35 (60)	0.45	0.36
Aldosterone antagonist	5 (9)	6 (10)	8 (14)	0.66	0.62
Beta blocker	40 (69)	42 (72)	45 (78)	0.57	0.80
Loop diuretic	36 (62)	43 (74)	51 (88)	0.006	0.013
Congestion and quality of life					
NT-proBNP, pg/mL (n=173)	382 (94–656)	696 (356–1621)	955 (497–1802)	<0.0001	<0.0001
Elevated JVP (n=168)	16 (28)	24 (44)	33 (59)	0.004	0.003
Moderate or severe edema	5 (9)	8 (14)	22 (38)	0.0006	0.0006
NYHA class II	35 (60)	26 (45)	24 (41)	0.09	0.029
MLHFQ score (n=166)	37 (30–63)	49 (35–65)	44 (25–60)	0.16	0.17

Values are median (interquartile range) or n (%). ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; COPD, chronic obstructive pulmonary disease; HF, heart failure; JVP, jugular venous pressure; MLHFQ, Minnesota Living with Heart Failure Questionnaire; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association.

*Adjusted for sex.

median level of ST2 in this HFpEF cohort was higher than that observed in studies of ambulatory HFpEF patients^{8,10} and approached the 33–35 ng/mL cut point associated with a higher risk of cardiovascular outcomes in chronic HFpEF.^{8,10,11} Median ST2 levels in RELAX were also higher than those reported in patients with ischemic heart disease (19–24 ng/mL)^{37,38} and in the general population (22 ng/mL)³⁴ but lower than those reported in acute HF or pulmonary disease (42–76 ng/mL)^{15,33,39} and much lower than observed in critically ill intensive care unit patients (555–745 ng/mL).^{33,40} Importantly, all of these studies used the same Presage[®] ST2 assay. Although relatively few studies have evaluated ST2 levels in HFpEF,^{6,18,35} the only

one to assess ST2 using this assay was a post hoc analysis of the PARAMOUNT (Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction) trial that described elevated median ST2 levels (33 ng/mL), similar to the HFpEF patients in RELAX.³⁵

Association of ST2 Levels With Clinical Characteristics

The associations we observed between ST2 levels and comorbidities including diabetes mellitus, renal dysfunction, and atrial fibrillation as well as congestion, diuretic use, and

Table 2. Exercise Performance by Tertile of Baseline ST2 Levels

	Low ST2 Tertile (n=58)	Mid ST2 Tertile (n=58)	High ST2 Tertile (n=58)	P Value	P Value*
Peak VO ₂ , mL/kg per minute	12.1 (10.9–14.4)	11.9 (10.8–15.1)	11.2 (10.0–13.3)	0.12	0.017
Peak VO ₂ , % predicted	44 (38–51)	42 (35–48)	39 (32–47)	0.024	0.017
Respiratory exchange ratio	1.11 (1.05–1.17)	1.09 (1.03–1.15)	1.09 (1.02–1.15)	0.41	0.41
Peak systolic BP, mm Hg	160 (140–184)	154 (138–168)	148 (126–168)	0.07	0.005
Rest HR, bpm	67 (59–76)	70 (60–75)	69 (61–79)	0.58	0.43
Peak HR, bpm	111 (96–136)	107 (91–120)	107 (91–128)	0.12	0.07
Chronotropic index	0.55 (0.38–0.71)	0.47 (0.29–0.62)	0.49 (0.28–0.69)	0.15	0.08
VE/VCO ₂ slope	31.7 (28.1–36.9)	33.8 (28.6–37.5)	34.9 (31.0–39.9)	0.06	0.20
6-minute walk distance, m	351 (265–434)	305 (244–372)	311 (230–360)	0.046	0.012

Total sample size with data (N=168–174). BP indicates blood pressure; bpm, beats per minute; HR, heart rate; VE/VCO₂, ventilatory efficiency; VO₂, oxygen consumption.

*Adjusted for sex.

New York Heart Association functional status have also been reported in studies of ambulatory patients with HFpEF^{8,10,11} and the PARAMOUNT HFpEF cohort.³⁵ Diabetes mellitus, renal dysfunction, atrial fibrillation, and chronic venous congestion are all linked to inflammation.⁴¹ It is perhaps surprising that obesity was not associated with ST2 levels in

this cohort or in the PARAMOUNT HFpEF cohort,³⁵ given a previous study showing higher ST2 levels in severe obesity and the known proinflammatory effect of obesity.⁴² However, the intensity and types of inflammation linked to activation of ST2 are not well defined.³³ It is important to note that ST2 levels are not associated with renal function in the general

Table 3. Baseline Cardiac Structure and Function by Tertiles of Baseline ST2 Levels

	N*	Low ST2 Tertile (n=58)	Mid ST2 Tertile (n=58)	High ST2 Tertile (n=58)	P Value	P Value†
Diastolic function parameters						
E/A ratio	118	1.4 (1.0–1.9)	1.3 (0.9–3.0)	1.6 (1.0–3.0)	0.70	0.68
Medial e', m/s	160	0.06 (0.04–0.07)	0.07 (0.05–0.08)	0.06 (0.05–0.08)	0.56	0.77
Medial E/e'	155	14.9 (11.3–22.0)	15.0 (10.0–20.0)	17.9 (13.1–24.5)	0.17	0.14
Deceleration time, ms	159	192 (159–215)	180 (158–219)	181 (151–219)	0.86	0.97
LA volume/BSA, mL/m ²	123	41 (33–50)	47 (34–62)	51 (35–62)	0.08	0.16
Left ventricular systolic function and geometry						
Ejection fraction, %	173	61 (57–66)	61 (56–66)	60 (55–63)	0.29	0.47
LVEDd/BSA, cm/m ²	133	2.3 (2.1–2.5)	2.2 (2.0–2.4)	2.2 (2.0–2.5)	0.58	0.91
Relative wall thickness	128	0.38 (0.34–0.44)	0.42 (0.36–0.52)	0.42 (0.37–0.46)	0.06	0.10
LV mass/BSA, g/m ²	128	76 (64–85)	72 (61–89)	80 (60–100)	0.98	0.60
Right ventricular load and function						
PASP, mm Hg	113	39 (32–48)	46 (34–58)	43 (32–51)	0.045	0.016
TAPSE, mm	172	19.0 (16.0–23.0)	17.5 (14.0–24.0)	16.0 (13.0–20.0)	0.013	0.015
Vascular function						
Systolic BP, mm Hg	174	128 (114–140)	123 (113–137)	124 (112–131)	0.44	0.25
Diastolic BP, mm Hg	174	70 (64–80)	70 (62–78)	69 (62–78)	0.66	0.50
Ao distensibility, 10 ⁻³ mm Hg ⁻¹	68	1.21 (0.67–1.46)	1.08 (0.58–2.25)	1.09 (0.67–1.76)	0.77	0.50

Data are median (interquartile range). Ao indicates aortic; BP, blood pressure; BSA, body surface area; LA, left atrial; LV, left ventricular; LVEDd, left ventricular end-diastolic dimension; PASP, pulmonary artery systolic pressure; TAPSE, tricuspid annular plane systolic excursion.

*Total sample with data.

†Adjusted for sex.

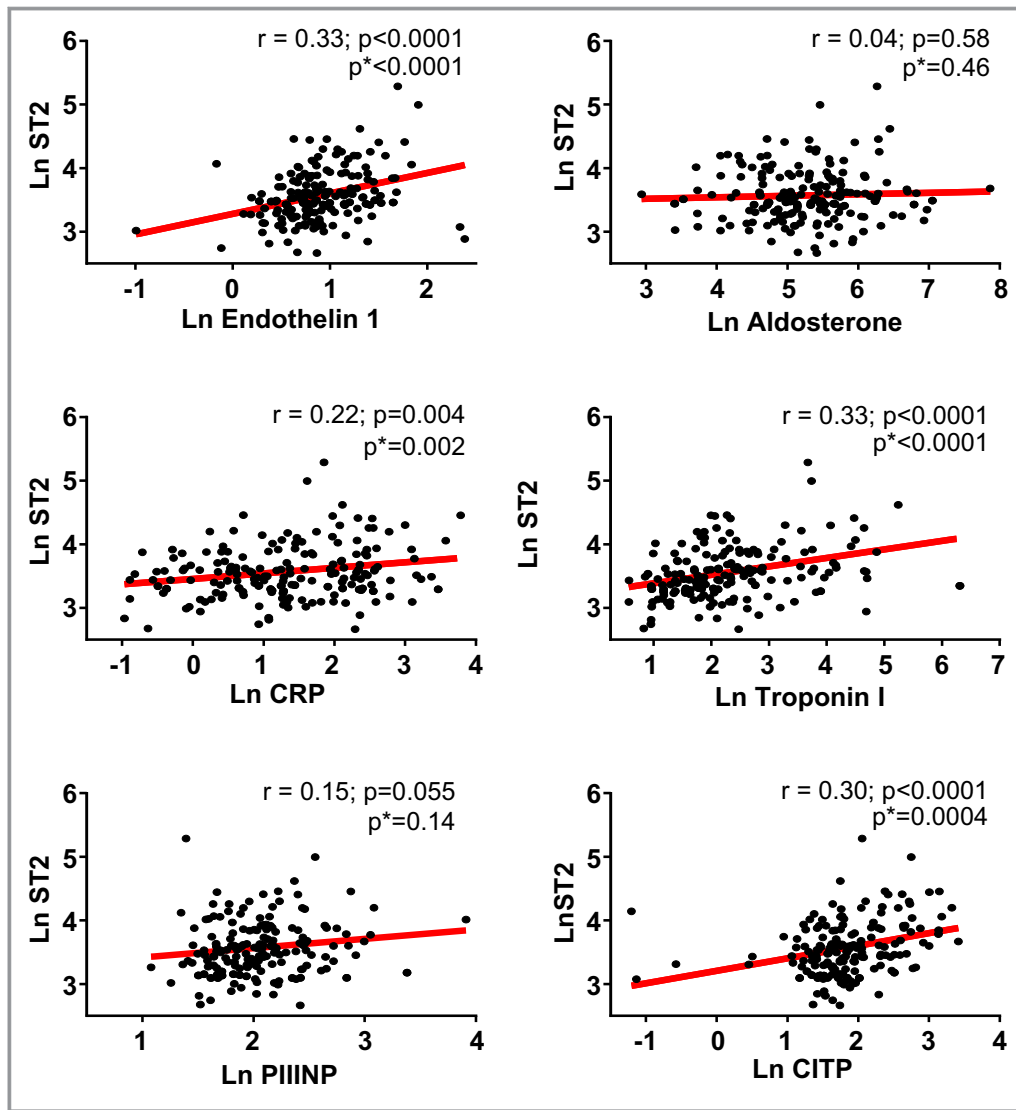


Figure 2. The relationship between ST2 and biomarkers in heart failure with preserved ejection fraction. ST2 was associated with endothelin 1, high-sensitivity C-reactive protein (CRP), C-telopeptide for type I collagen (C1TP), and troponin I but not aldosterone or pro-collagen III N-terminal peptide (PIINP) levels. *Adjusted for sex. Ln indicates log transformed.

population³⁴ or elevated in a small sample of chronic kidney disease patients without HF,³³ suggesting a unique interaction among HF, renal dysfunction, and ST2 levels.

Association of ST2 Levels With Functional Capacity

In HFpEF, as in HFReEF, ST2 levels were associated with worse exercise capacity. Because skeletal muscle function is a potent determinant of peak oxygen consumption in HFpEF,²⁵ this association may reflect effects of systemic inflammation on skeletal muscle or peripheral vascular function, given the lack of association of ST2 and resting LV structure and function.

Association of ST2 Levels With Cardiac Structure and Function

Although an association between ST2 and ventricular dysfunction was demonstrated in animal studies,⁴ a relationship between circulating ST2 levels and the severity of cardiac remodeling or dysfunction in clinical HF is less clear.^{8,35,43–45}

In general population cohorts with a spectrum of LV geometry and function, ST2 levels were not associated with LV mass⁴⁶ or ejection fraction^{47,48} but were associated with a greater likelihood of asymptomatic diastolic dysfunction in one study.⁴⁷ In a diverse cohort of patients evaluated in an emergency department for dyspnea and subsequently found to have HFReEF, HFpEF, or noncardiac dyspnea, ST2 levels were

higher in HF than noncardiac dyspnea and, across the combined HF groups, were associated with LV ejection fraction, RV systolic dysfunction, higher pulmonary artery pressures, and more severe tricuspid regurgitation but not with LV mass or diastolic function.⁴³ In another study of patients with acute dyspnea and normal ejection fraction (most with noncardiac dyspnea), ST2 was not associated with LV mass or most Doppler measures of diastolic function.⁴⁴ ST2 levels were not meaningfully associated with LV remodeling in HFREF.⁸ The PARAMOUNT analysis in HFpEF found a modest association of ST2 levels with left atrial volumes but no other parameter of cardiac structure or function, although RV indices were not assessed.³⁵

In the RELAX HFpEF cohort, we found no association between LV structure or function and ST2 levels, whereas NT-proBNP (of myocardial origin²⁰) was strongly associated with cardiac structure and function. The association of ST2 levels with RV function observed in this study may be related to the impact of RV dysfunction on systemic venous congestion and its known proinflammatory effects.²⁹

Mechanism of ST2 Activation in HFpEF

We did not measure the transcardiac ST2 gradient, and thus the systemic versus cardiac source of elevated levels of ST2 in HFpEF cannot be established in our study. The lack of a relationship between ST2 and LV structure and function and the associations between ST2 and proinflammatory comorbidities, severity of congestion, and biomarkers reflective of systemic inflammation and fibrosis (high-sensitivity C-reactive protein and C-telopeptide for type I collagen) and myocardial injury or inflammation (high-sensitivity troponin I) may implicate an extracardiac source of ST2 in HFpEF, as has been established in HFREF.^{17–20} Indeed, human saphenous venous and arterial endothelial cells exposed to inflammatory stimuli have been shown to secrete ST2,^{17,18,49} and we speculate that that activation of ST2 reflects systemic inflammation in HFpEF. Whether the inflammatory stimulus putatively responsible for ST2 activation is mediated by comorbidity-driven microvascular endothelial inflammation or the systemic congestion and end-organ hypoperfusion common to advanced HFpEF or HFREF is deserving of further study.

Limitations

Given the post hoc nature of this study and its modest sample size, this analysis was deemed to be exploratory with the aim of generating hypotheses that could be tested in larger mechanistic HFpEF studies. The RELAX cohort had relatively advanced HFpEF,^{30,50} and this may limit correlations, although the range of ST2 levels was fairly broad. Those

patients missing stored serum appeared to have more severe HF; some associations may have been stronger if the entire cohort had available serum. The association of ST2 levels and outcomes was not assessed in RELAX, given the short duration of follow-up.

Conclusion

In HFpEF, ST2 levels were elevated and associated with proinflammatory comorbidities; RV pressure overload and dysfunction; systemic congestion; exercise intolerance; and biomarkers reflective of systemic inflammation and fibrosis, neurohumoral activation, and myocardial injury but not with LV structure and function. These data add to our understanding of ST2 as a biomarker in HFpEF and suggest that systemic inflammation may mediate or contribute to activation of ST2 in HFpEF.

Sources of Funding

This study was supported by grants from the National Heart, Lung, and Blood Institute: U01HL084904 (for the data coordinating center), and U01HL084861, U01HL084875, U01HL084877, U01HL084889, U01HL084890, U01HL084891, U01HL084899, U01HL084907, and U01HL084931 (for the clinical centers). This study was not supported by industry.

Disclosures

Dr Felker consults for Singulex, Roche Diagnostics, and receives grant support from the National Institutes of Health and Roche Diagnostics. Dr Chen reports that he and Mayo Clinic have patented and licensed designer natriuretic peptides to Anexon Inc and Capricor Therapeutics. Dr Chen is the cofounder of Zumbro Discovery. Dr Braunwald receives grant support to his institution from Duke University for his role as chair of the National Heart, Lung, and Blood Institute–sponsored Heart Failure Network. All others report no disclosures relevant to this manuscript.

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SUPPLEMENTAL MATERIAL

Table S1. Characteristics of patients with or without stored serum for ST2 levels

	Have ST2 (n=174)	Missing ST2 (n=42)	p-value
Male	86 (49)	26 (62)	0.15
Age, y	68 (62 - 77)	70 (62 - 80)	0.41
Body Mass Index, Kg/m ²	32.9 (28.4 – 38.8)	33.6 (27.9 – 40.9)	0.79
Body Surface Area, m ²	2.11 (1.94 - 2.29)	2.10 (1.88 - 2.33)	0.74
HF hospitalization	55 (32)	24 (57)	0.002
Comorbidities			
Hypertension	146 (84)	37 (88)	0.50
Ischemic heart disease	67 (39)	17 (40)	0.81
Atrial fibrillation	85 (49)	26 (62)	0.13
COPD	32 (18)	10 (24)	0.43
Diabetes mellitus	71 (41)	22 (52)	0.17
Creatinine, mg/dl	1.1 (0.8 - 1.3)	1.3 (0.9 - 1.7)	0.005
Cystatin-C, mg/l	1.25 (1.04 - 1.66)	1.65 (1.19 - 1.95)	0.005
Medications			
ACE inhibitor or ARB	116 (67)	36 (86)	0.015
Aldosterone antagonist	19 (11)	4 (10)	1.00
Beta blocker	127 (73)	37 (88)	0.039
Loop diuretic	130 (75)	36 (86)	0.13
Congestion and Quality of Life			
NT-proBNP, pg/ml	633 (219-1372)	1334 (499 – 2350)	0.004
Elevated JVP (n=209)	73 (43)	22 (54)	0.69
≥ Moderate edema	35 (20)	9 (21)	0.45
NYHA class II	85 (49)	16 (38)	0.21
MLHFQ Score	43 (30 - 63)	45 (22 - 58)	0.69

Abbreviations: ACE, Angiotensin Converting Enzyme; ARB, Angiotensin Receptor Blocker; COPD, Chronic Obstructive Pulmonary Disease; HF, Heart Failure; NT-proBNP, N-terminal pro-brain natriuretic peptide; JVP, Jugular Venous Pressure; MLHFQ, Minnesota Living with Heart Failure Questionnaire; NYHA, New York Heart Association

Table S2. Association of ST2 with LV systolic function and geometry by cardiac magnetic resonance imaging

	N*	Low ST2 Tertile (n=58)	Mid ST2 Tertile (n=58)	High ST2 Tertile (n=58)	p-value	p- value†
CMRI						
Ejection fraction, %	96	67 (62 - 71)	65 (58 - 70)	67 (53 - 70)	0.54	0.53
LVEDV / BSA, ml/ m ²	96	54 (46 - 63)	55 (43 - 64)	53 (46 - 66)	1.00	0.93
LV mass/LV volume, g/ml	96	1.05 (0.87 - 1.31)	1.23 (1.00 - 1.71)	1.17 (0.99 - 1.32)	0.07	0.36
LV mass/BSA, g/m ²	96	56 (52 - 65)	69 (53 - 84)	62 (56 - 79)	0.06	0.29

Data are median (IQR); * total N with data; † Adjusted for sex

Abbreviations: LV, Left Ventricle; LVEDV, Left Ventricular End Diastolic Volume

Table S3. Association of NT-proBNP levels with cardiac structure and function

	NT-proBNP*		
	N	Parameter estimate per log unit	Unadjusted p-value
<i>Diastolic function parameters</i>			
Log-transformed E/A ratio	117	0.21637	<0.0001
Log-transformed Medial E/e'	154	0.16177	<0.0001
Deceleration time, ms	158	-9.28482	0.0004
LA volume/BSA, ml/m ²	122	6.99614	<0.0001
<i>LV structure and systolic function</i>			
Ejection fraction	172	-0.32805	0.45
Log-transformed relative wall thickness	127	0.04831	0.003
LV mass/BSA, g/m ² (echo)	127	3.94840	0.041
<i>Right ventricular load and function</i>			
PASP, mmHg	112	2.70231	0.005
TAPSE, mm	171	-2.03747	<0.0001

* Linear regression model with the imaging measure as the dependent variable and log-transformed NT-proBNP as the independent variable.

Abbreviations: LA, Left Atrium; LV, Left Ventricle; PASP, Pulmonary Artery Systolic Pressure; TAPSE, Tricuspid Annular Plane Systolic Excursion