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## Soluble ST2 in Ambulatory Patients with Heart Failure: Association with Functional Capacity and Long-Term Outcomes

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### Abstract

**Background**—ST2 is involved in cardioprotective signaling in the myocardium and has been identified as a potentially promising biomarker in HF. We evaluated ST2 levels and their association with functional capacity and long-term clinical outcomes in a cohort of ambulatory heart failure (HF) patients enrolled in the HF-ACTION study—a multicenter, randomized study of exercise training in HF.

**Methods and Results**—HF-ACTION randomized 2331 patients with left ventricular ejection fraction <0.35 and New York Heart Association class II–IV HF to either exercise training or usual care. ST2 was analyzed in a subset of 910 patients with evaluable plasma samples. Correlations and Cox models were used to assess the relationship among ST2, functional capacity, and long-term outcomes.

The median *baseline* ST2 level was 23.7 ng/mL (interquartile range, 18.6–31.8). ST2 was modestly associated with measures of functional capacity. In univariable analysis, ST2 was significantly associated with death or hospitalization (hazard ratio [HR], 1.48;  $p < 0.0001$ ), cardiovascular death or HF hospitalization (HR, 2.14;  $p < 0.0001$ ), and all-cause mortality (HR, 2.33;  $p < 0.0001$ ) (all HRs for  $\log\text{-base}^2$  ng/mL). In multivariable models, ST2 remained

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independently associated with outcomes after adjustment for clinical variables and amino-terminal pro-B-type natriuretic peptide. However, ST2 did not add significantly to reclassification of risk as assessed by changes in the C statistic, net reclassification improvement, and integrated discrimination improvement.

**Conclusions**—ST2 was modestly associated with functional capacity and was significantly associated with outcomes in a well-treated cohort of ambulatory HF patients, although it did not significantly affect reclassification of risk.

### Keywords

heart failure; biomarker; prognosis

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### Introduction

Chronic heart failure is a major and growing public health issue, affecting more than 5 million people in the United States. Although there has been substantial progress in heart failure therapy, overall prognosis remains poor, with 5-year mortality approaching 50% in symptomatic patients<sup>1, 2</sup>. At the same time, the available treatment options have proliferated and include many expensive and complex treatments such as cardiac resynchronization therapy, left ventricular assist devices, and invasive hemodynamic monitors. Risk stratification is therefore increasingly critical in order to apply costly and invasive therapies to those patients most likely to benefit.

Biomarkers, in particular the natriuretic peptides, play an increasingly important role in the risk stratification of heart failure patients<sup>3</sup>. A variety of biomarkers have emerged as potentially useful clinical tools for heart failure, including ST2<sup>4</sup>, galectin-3<sup>5</sup>, growth-differentiation factor 15<sup>6</sup>, copeptin<sup>7</sup>, and midregional pro-hormone adrenomedullin<sup>8</sup> among many others. There remains a need to validate these newer biomarkers in diverse populations with careful covariate adjustment in order to clarify their role in heart failure management.

ST2 has been identified as the ligand for interleukin-33 (IL-33) and exists in both a trans-membrane and soluble form. IL-33/ST2 signaling in the myocardium appears to be a cardioprotective mechanism that limits hypertrophy and myocardial fibrosis<sup>9</sup>. Circulating ST2 appears to function as a “decoy receptor,” modulating the effects of excessive IL-33 signaling<sup>10</sup>. The soluble form of ST2 has recently been identified as a promising biomarker in patients with both acute coronary syndromes<sup>11</sup> and heart failure<sup>4, 12</sup>. Accordingly, we sought to evaluate how circulating ST2 levels are associated with other measures of heart failure status and with long-term clinical outcomes in a cohort of ambulatory heart failure patients enrolled in the Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training (HF-ACTION) study—a large, multicenter, randomized study of exercise training in heart failure.

## Methods

The primary results, design and rationale of the HF-ACTION study have been previously published<sup>13, 14</sup>. Briefly, HF-ACTION (ClinicalTrials.gov identifier: NCT00047437) was a randomized clinical study evaluating the effects of exercise training vs. usual care on long-term morbidity and mortality in patients with chronic heart failure due to left ventricular systolic dysfunction (New York Heart Association [NYHA] class II–IV, left ventricular ejection fraction [LVEF]<0.35). The primary endpoint of HF-ACTION was a composite of all-cause mortality and all-cause hospitalization. Randomization to *exercise training in the HF-ACTION study was associated with modest reductions in the primary endpoint and other cardiovascular endpoints*. An independent clinical events committee adjudicated all deaths and first cardiovascular hospitalizations. HF-ACTION was approved by local institutional review boards, and all enrolled patients provided written informed consent.

### Biomarker assays

A subset of patients enrolled in the HF-ACTION study who agreed to participate in the biomarker substudy underwent plasma collection at baseline, 3 months, and 12 months. Baseline blood samples were obtained on the same day as baseline cardiopulmonary exercise (CPX) testing but prior to exercise. Samples were collected via peripheral vein into EDTA-containing tubes, centrifuged immediately, and then stored at  $-70^{\circ}\text{C}$  for subsequent analysis. Soluble ST2 levels were assessed on baseline samples using a highly sensitive sandwich monoclonal immunoassay (Presage<sup>®</sup> ST2 Assay, Critical Diagnostics, New York, NY), with a lower limit of detection of 2 ng/mL, an upper limit of detection of 200 ng/mL, an intra-assay coefficient of variation <2.5%, and an interassay coefficient of variation of <4.6%<sup>15</sup>. Amino-terminal pro-B-type natriuretic peptide (NT-proBNP) was assessed at a central core lab using a clinically available assay (Roche Elecsys<sup>®</sup>, Roche Diagnostics, Indianapolis, IN). The core laboratories were blinded to all clinical data.

### Statistical analysis

Baseline characteristics were described using medians and interquartile ranges or proportions. The outcome variables of interest were 1) time to all-cause hospitalization or all-cause mortality (the primary outcome of the HF-ACTION study), 2) time to cardiovascular death or heart failure hospitalization, 3) time to all-cause mortality, and 4) change in peak  $\text{VO}_2$  from baseline to 3 months.

ST2 was a continuous variable in all models but was log transformed for analysis because it was not normally distributed. Hazard ratios (HRs) were calculated for the log base 2, such that the reported HR represented the risk per doubling of ST2. Although our primary analysis was focused on ST2 as a continuous variable, for descriptive purposes we also examined ST2 as a dichotomous variable above and below the clinical cutpoint of 35 ng/mL (based on receiver operating characteristic curve analysis and U.S. Food and Drug Administration labeling)<sup>4</sup>.

The relationships among ST2 levels and other baseline variables of interest were analyzed using simple correlations. For the clinical outcomes of interest, we analyzed the

relationships using a series of Cox proportional hazards models, including adjusting for demographics alone (age, sex, race) *as well as additional adjustment* for the more comprehensive set of predictors (“final clinical model”) that were identified in the adjustment model developed for the overall HF-ACTION cohort for each endpoint *as previously described*<sup>16</sup>. (see Appendix for a list of covariates). For the relationship between baseline ST2 and change in peak VO<sub>2</sub> at 3 months, inverse probability weighting was used to adjust for missingness of exercise parameters during follow-up (16% of patients with available ST2 data were missing peak VO<sub>2</sub> at 3 months). The relationship between ST2 at baseline and change peak VO<sub>2</sub> at 3 months was then analyzed using a linear regression model that included change in peak VO<sub>2</sub> at 3 months (transformed to achieve normality) as the response variable and ST2 (log<sub>2</sub> ST2) along with potential confounders as explanatory variables.

Although NT-proBNP was not part of the modeling process for the overall HF-ACTION study because it was not available in the entire cohort, we additionally examined each model with and without NT-proBNP, given its known strong association with outcomes in chronic heart failure<sup>17</sup>. Because the final adjustment model from the overall HF-ACTION dataset included variables from CPX testing, which is not routinely available in some clinical settings, we examined each adjusted model with and without CPX variables.

We examined ST2’s added value to existing models by comparing model discrimination and risk prediction between models with and without log<sub>2</sub> ST2. The specific measures that we considered were the C statistic, the integrated discrimination improvement (IDI), and the net reclassification improvement (NRI). For each model comparison, the summary measures were reported along with 95% bootstrap confidence intervals based on 100 replications. We calculated NRI using the Kaplan-Meier estimates of event probabilities at the end of study follow-up. Because there are no widely accepted categories for the absolute risk of HF, we calculated the NRI without categories.

Finally, to further explore the *potential relationship* between NT-proBNP and ST2, we evaluated the risk in 4 groups defined by dichotomizing each variable (ST2 as above or below 35 ng/mL cutpoint and NT-proBNP at the median = 857 ng/ml). A p-value 0.05 was considered statistically significant for all analyses. All analyses were completed using SAS 9.2 (Cary, NC).

## Results

Evaluable baseline plasma samples were available for 910 patients. Baseline characteristics for the entire HF-ACTION cohort and the subset with ST2 data available, further stratified by ST2 above and below 35 ng/mL, are shown in Table 1. The median age of the study cohort was 59 years; 64% were white, and 71% were male. The median NT-proBNP level was 857 pg/mL, median LVEF was 24%, and median serum creatinine was 1.2 mg/dL. Utilization of guideline-based medical therapy for systolic heart failure was high, with 95% receiving beta-blockers and 74% receiving angiotensin-converting enzyme (ACE) inhibitors. The subset of patients with available plasma samples for analysis (n=910) was broadly similar to the HF-ACTION cohort as a whole (n=2331, see Table 1), except that the

biomarker substudy was limited to patients enrolled in North America. The median ST2 level was 23.7 ng/mL (IQR, 18.6–31.8), and 19.6% (n=179 of 910) of patients had ST2 levels above the proposed prognostic cutpoint of 35 ng/mL. Patients with elevated ST2 levels were more likely to be men and to have markers of more advanced disease, including higher NYHA class, higher NT-proBNP, and worse renal function ( $p<0.001$  for all).

### ST2, functional capacity, and quality of life

We assessed the relationships between ST2 and physician-assessed functional capacity (NYHA class), objective measures of maximal functional capacity (maximal oxygen consumption [peak  $\text{VO}_2$ ]) and submaximal functional capacity (6-minute walk distance), and patient-reported quality of life (Kansas City Cardiomyopathy Questionnaire [KCCQ] summary score). ST2 levels progressively increased with worsening NYHA class (Figure 1). In general, higher log ST2 levels were significantly but modestly correlated with poorer baseline functional capacity, including peak  $\text{VO}_2$  on CPX testing ( $r = -0.30$ ;  $p<0.001$ ) and 6-minute walk distance ( $r = -0.22$ ;  $p<0.001$ ). Higher levels of ST2 were modestly associated with lower quality of life summary scores for the KCCQ ( $r = 0.15$ ;  $p<0.001$ ). When ST2 was considered as a dichotomous variable, an ST2 level  $>35$  ng/mL was associated with significantly worse peak  $\text{VO}_2$ , CPX exercise duration, and 6-minute walk distance ( $p<0.001$  for all).

### ST2 and long-term outcomes

The study cohort was followed for 32 months, during which the raw events rates were 171 deaths (19%), 646 all-cause deaths or hospitalizations (71%), and 312 cardiovascular deaths or heart failure hospitalizations (34%). In univariable analysis, ST2 was a significantly associated with all 3 long-term outcomes. Specifically, doubling of the baseline ST2 level was associated with a significantly increased risk of all-cause death or hospitalization (HR, 1.48;  $p<0.0001$ ), cardiovascular death or heart failure hospitalization (HR, 2.14;  $p<0.0001$ ), and all-cause mortality (HR, 2.33;  $p<0.0001$ ) (Figure 2). When considered along with NT-proBNP as 4 groups based on NT-proBNP levels above or below the median and ST2 levels above or below the 35 ng/mL cutpoint, patients with both markers elevated had a 5.6-fold increased risk of death during the course of long-term follow up compared to those with neither marker elevated. (Figure 3). There was no statistical interaction between ST2 and NTproBNP ( $p = 0.44$ ).

To adjust for how other covariates may affect the association between ST2 and outcome, we constructed a series of multivariable models (Table 2). Models were progressively adjusted for demographics alone, demographics + clinical variables, and demographics + clinical variables + CPX variables. Finally, each step was assessed with and without the inclusion of NT-proBNP. For each endpoint, ST2 remained independently associated with long-term outcomes after adjusting for demographics and NT-proBNP. For the endpoint of cardiovascular death + heart failure hospitalization, ST2 remained statistically associated (adjusted HR, 1.32 per doubling of ST2;  $p=0.0129$ ) even after adjusting for demographics, NT-proBNP, and clinical and CPX variables. For all-cause mortality, the final adjusted HR was similar (1.26), though it did not reach nominal statistical significance ( $p=0.0877$ ). ST2 was not significantly associated with the composite of all-cause mortality +

rehospitalization (adjusted HR, 1.08;  $p=0.3605$ ) after final adjustment for demographics, NT-proBNP, and the final clinical model, including CPX variables.

Because CPX testing is infrequently used or is unavailable at some centers, we examined the predictive value of ST2 when adjusted for clinical variables and NT-proBNP but *excluded CPX variables (peak  $VO_2$ , exercise duration, and  $VE-VCO_2$  slope)*. In this analysis, ST2 was significantly associated with all-cause mortality (adjusted HR, 1.42;  $p=0.0067$ ) and cardiovascular death or heart failure hospitalization (adjusted HR, 1.37;  $p=0.0037$ ), but it was not with all-cause mortality or hospitalization (adjusted HR, 1.13;  $p=0.1213$ ). To evaluate the incremental value of ST2, we examined several measures of model performance, including the change in the C statistic, IDI and category free NRI. The addition of ST2 did not substantially change the C-statistic, IDI, or NRI for any of the clinical endpoints (Table 3). This suggests that while ST2 is significantly associated with outcomes, adding ST2 to models containing the clinical risk factors and NT-proBNP does not incrementally improve discrimination or risk prediction.

### ST2 and response to exercise training

Given that HF-ACTION was a randomized, controlled study of a unique intervention (exercise training), we tested for an interaction between baseline ST2 levels and the treatment effect of exercise training with regard to each of the clinical outcomes of interest. Of the endpoints tested, there was a statistical interaction between exercise training and the outcome of all-cause mortality, such that patients with lower ST2 levels were more likely to have a benefit of exercise training than were patients with higher levels ( $p=0.016$  for ST2 \* treatment interaction), and a similar interaction for cardiovascular mortality ( $p = 0.032$  for ST2\* treatment interaction). There was not a similar statistical interaction between ST2 levels and treatment effects with regard to the composite endpoints of all-cause death or hospitalization ( $p=0.620$ ) or cardiovascular death or heart failure hospitalization ( $p=0.224$ ).

We also assessed the relationship between baseline ST2 levels and the change in peak  $VO_2$  between baseline and 3 months. Higher baseline ST2 levels were associated with a greater training effect (i.e., the increase in peak  $VO_2$  from baseline to 3 months (adjusted  $p$  value = 0.034)). There was no significant interaction between baseline ST2 levels and treatment assignment (exercise vs. control) (Figure 4).

## Discussion

Soluble ST2 is a novel biomarker that reflects a variety of important pathologic processes in heart failure, including myocyte stretch and inflammation. ST2/IL-33 signaling is induced by myocyte stretch and modulates hypertrophic responses and fibrosis in the myocardium<sup>18</sup>. Intact ST2/IL-33 signaling is believed to be a mechanically activated cardioprotective paracrine signaling system that shields the myocardium from the adverse effects of overload. Because ST2 elevations reflect activation of distinct biologic systems compared with the natriuretic peptides, there is significant interest in whether ST2 can add additional prognostic information. In this analysis of the HF-ACTION study, ST2 was significantly associated with long-term outcome even after an adjustment for clinical covariates. *Addition of NT-proBNP attenuated the association with the primary outcome of all-cause death and*

*hospitalizations*. To our knowledge, this is the most robust test of this novel biomarker to date with regard to covariate adjustment in a large, multicenter cohort of ambulatory heart failure patients. Although the association between ST2 and outcomes was consistent across all outcomes, the addition of ST2 to a model adjusted for known risk factors did not lead to improved discrimination and risk prediction based on reclassification analysis. The overall effect sizes were modest (all adjusted HR were < 1.5 per doubling of ST2). These data demonstrate the limitations of multivariable modeling alone as an assessment of the clinical value of new biomarkers.

We believe these findings are of interest for several reasons. To our knowledge, this is the first study to have comprehensively examined the relationship between ST2, commonly used measures of functional capacity, and response to exercise training in heart failure patients. We found that ST2 levels were significantly associated with both physician assessed (NYHA class) and objective measures of functional capacity (6-minute walk distance and peak  $\text{VO}_2$ ). Additionally, baseline levels of ST2 were predictive of training effect (the change in peak  $\text{VO}_2$  over 3 months). We also identified an interaction between ST2 levels and exercise training, such that patients with lower ST2 levels were more likely to have beneficial effects of exercise training with regard to the endpoint of all-cause mortality. We did not observe a similar interaction for the composite endpoints, and it is uncertain whether this observation represents a true effect or is a due to chance. Prior data have suggested a relationship between extracellular matrix turnover and changes in exercise capacity over time<sup>19</sup>. Weir et al showed that high ST2 levels are associated with greater response to eplerenone therapy after acute myocardial infarction<sup>20</sup>. Recent data regarding galectin-3, another marker thought to reflect myocardial fibrosis and remodeling, have suggested a similar differential effect (greater efficacy of statin therapy in patients with lower levels of the biomarker)<sup>21</sup>. Further analyses in other randomized trial datasets will be needed to confirm or refute this initial signal regarding the interaction between ST2 levels and treatment response in heart failure.

Our study extends the results of other studies that have examined the relationship between ST2 and outcomes in chronic heart failure<sup>4, 22, 23</sup>. Most notably, our data complement the previous analysis from Ky et al from the Penn Heart Failure Study (PHFS), which showed an association between ST2 levels and outcomes in an observational cohort from 3 tertiary care referral centers<sup>4</sup>. In comparison, our study cohort was more broadly representative of the systolic heart failure population because we included both a variety of academic- and community-based clinical sites and had a more representative proportion of patients with ischemic etiology (51% for HF-ACTION vs. 31% for PHFS). Additionally, our study had blinded independent adjudication of clinical outcomes, allowing for the robust distinction between disease-specific and all-cause endpoints. We found that the relationship between ST2 and outcomes may be stronger for disease-specific outcomes (such as HF hospitalization and CV mortality) compared with all-cause outcomes. HF-ACTION routinely collected detailed data on functional capacity using CPX testing and 6-minute walk distance, allowing for both an assessment of the relationship between ST2 and functional capacity as well as more detailed covariate adjustment in multivariable models. Finally, because HF-ACTION was an intervention study, we were able to assess interactions between the

intervention of exercise training (which has putative immunomodulatory effects)<sup>24</sup> and ST2 levels with regard to outcomes.

### Limitations

The study population for this analysis was derived from the HF-ACTION study, and as such, is susceptible to the limitations inherent in clinical trial populations. Although more broadly generalizable than other cohorts in which ST2 has been examined, our population was still relatively young (median age 59) compared with the broader heart failure population. *HF-ACTION was a randomized study and the study intervention (exercise training) did demonstrate a treatment effect, a fact that may have potentially influenced our results and could limit the generalizability to other settings.* Our study population consisted only of patients with impaired ejection fraction (LVEF <0.35), so our results cannot be extrapolated to the population of patients with heart failure and preserved ejection fraction.

### Conclusions

In this analysis of a large clinical study cohort of well-treated ambulatory heart failure patients, elevation of ST2 was significantly associated with long-term outcomes, especially disease-specific outcomes. These associations were relatively robust for disease specific endpoints (cardiovascular death and heart failure hospitalization) in traditional multivariable modeling, persisting even after comprehensive covariate adjustment, including demographics, clinical variables, and NT-proBNP. Despite this robust relationship in traditional multivariable modeling, ST2 did not add significantly to discrimination or risk prediction. Ongoing research will further clarify the role of ST2 in risk stratification in chronic heart failure.

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### Abbreviations

<b>ACE</b>	angiotensin-converting enzyme
<b>CPX</b>	cardiopulmonary exercise test
<b>HR</b>	hazard ratio
<b>IL-33</b>	interleukin-33
<b>KCCQ</b>	Kansas City Cardiomyopathy Questionnaire
<b>LVEF</b>	left ventricular ejection fraction
<b>NT-proBNP</b>	amino-terminal pro-B-type natriuretic peptide
<b>NYHA</b>	New York Heart Association
<b>Peak VO<sub>2</sub></b>	maximal oxygen consumption



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## Appendix

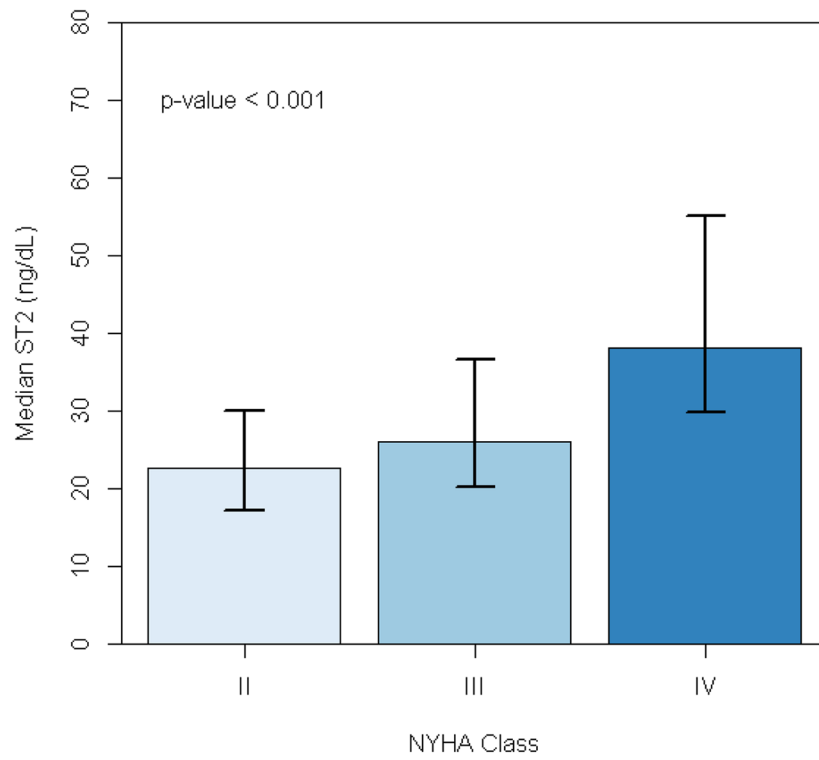
Final covariates used in adjusted models for each endpoint:

*All-cause death or all-cause hospitalization (primary endpoint):* peak VO<sub>2</sub> (as Weber class), sex, region (United States vs. non-United States), mitral regurgitation severity, ventricular conduction on electrocardiogram, blood urea nitrogen, ejection fraction, beta-blocker dose (truncated at 50 mg/day carvedilol equivalent), and Kansas City Cardiomyopathy Questionnaire symptom stability score.

*Cardiovascular death or heart failure hospitalization:* sex, race, age (truncated above 62 years), loop diuretic dose (using furosemide equivalent, truncated above 100 mg/day), left

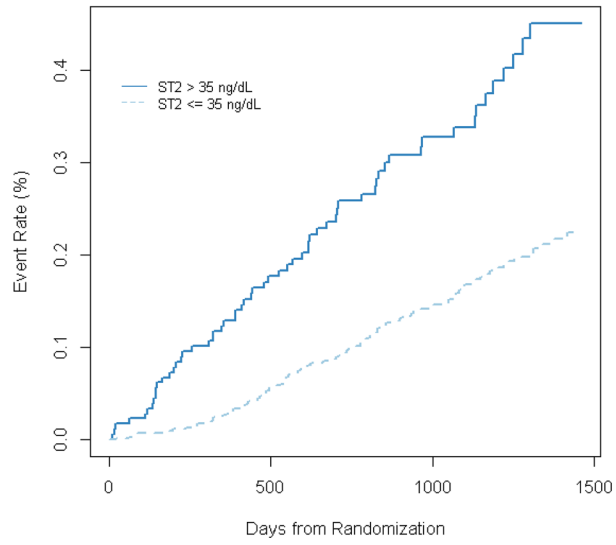
ventricular ejection fraction, mitral regurgitation severity, blood urea nitrogen (truncated above 39 mg/dL), VE/VC<sub>02</sub> slope (truncated above 80), Kansas City Cardiomyopathy Questionnaire symptom stability score, ventricular conduction on electrocardiogram, baseline maximal oxygen consumption, truncated above 28 mL/kg/min.

*All-cause mortality:*sex, body mass index (truncated above 25), loop diuretic dose (truncated above 100mg furosemide daily), Canadian Angina Class, ventricular conduction on electrocardiogram, left ventricular ejection fraction, exercise duration on cardiopulmonary exercise test, serum creatinine (truncated above 2.3).

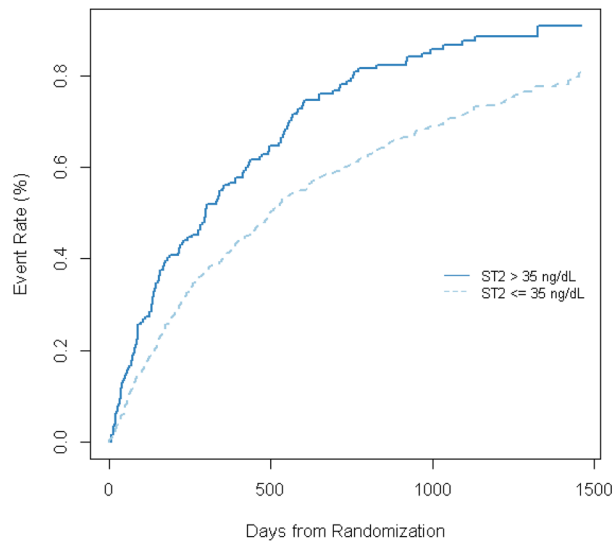


**Figure 1.** Median ST2 levels by NYHA class. Error bars represent intraquartile range.

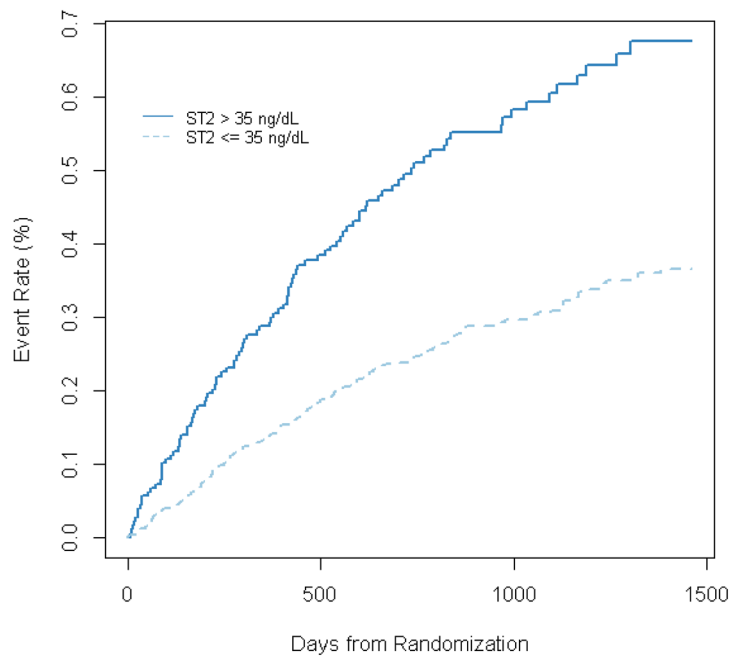
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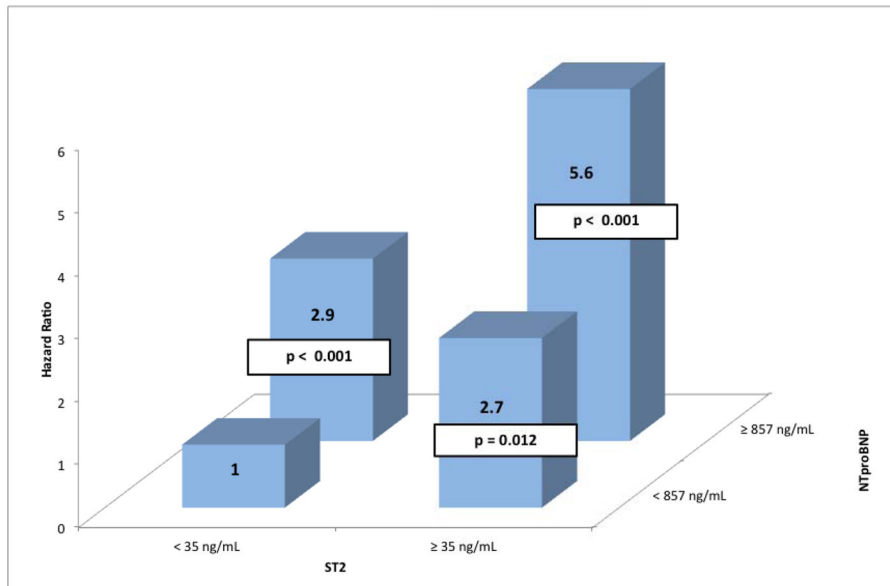
B.



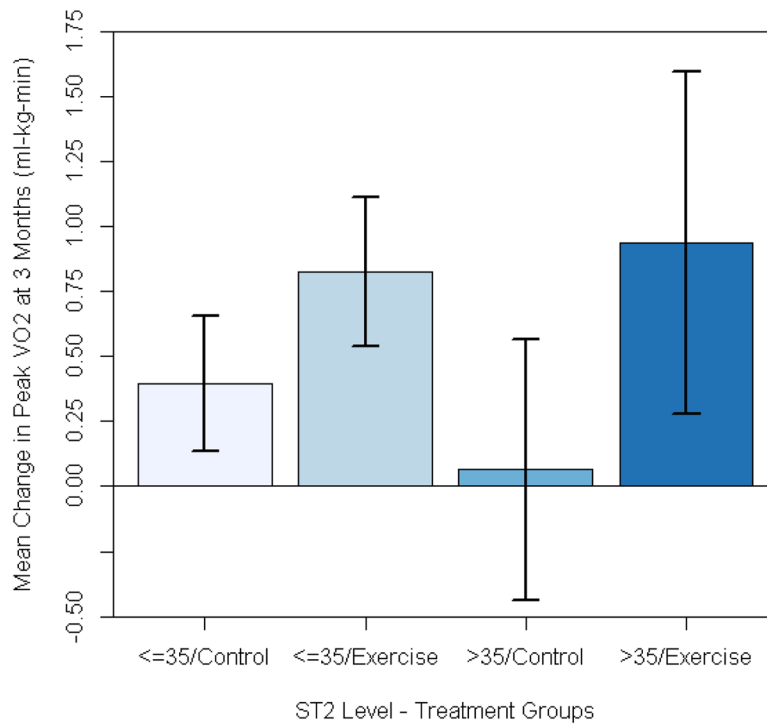
C)



**Figure 2.** Kaplan Meier plot for ST2 above or below 35 ng/mL for (A) All cause mortality, (B) all-cause mortality+ all cause hospitalization, and C) CV death + Heart failure hospitalization



**Figure 3.** Hazard for all-cause mortality based on groups defined by NT-proBNP (above or below median of 857 pg/mL) and ST2 (above or below 35 ng/mL), compared with a reference group with low NT-proBNP and low ST2. P values are for change relative to reference group. Interaction between NTproBNP and ST2 was not significant (p = 0.44).



**Figure 4.** Mean change from baseline to 3 months in peak VO<sub>2</sub> stratified by treatment assignment and ST2 levels. Baseline ST2 level was significant predictor of change in peak VO<sub>2</sub> (adjusted p = 0.034). There was no significant interaction between treatment assignment and ST2 levels with regard to predicting improvement in peak VO<sub>2</sub> (p = 0.67 for treatment\*ST2 interaction). Error bars represent standard deviations.



**Table 1**

Baseline Characteristics by ST2 Levels

Variable	Total Population (N=2331)	Substudy Population (N=912)	ST2 35ng/mL (N=731)	ST2 > 35ng/mL (N=179)	P-Value
Randomized treatment (exercise)	1159/2331 (49.7%)	453/912 (49.7%)	364/731 (49.8%)	87/179 (48.6%)	0.775
Age, y	59.3 (51.1,68.0)	59.2 (50.6,68.4)	58.5 (50.0,67.5)	63.3 (55.1,71.9)	<.001
Sex, % woman	661/2331 (28.4%)	264/910 (29.0%)	235/731 (32.1%)	29/179 (16.2%)	<.001
Race (%Caucasian)	1426/2296 (62.1%)	578/898 (64.4%)	458/721 (63.5%)	120/177 (67.8%)	0.525
History of diabetes	748/2331 (32.1%)	299/910 (32.9%)	230/731 (31.5%)	69/179 (38.5%)	0.071
History of MI	979/2331 (42.0%)	375/910 (41.2%)	284/731 (38.9%)	91/179 (50.8%)	0.003
History of hypertension	1388/2318 (59.9%)	578/906 (63.8%)	464/728 (63.7%)	114/178 (64.0%)	0.939
Current Smoker	388/2320 (16.7%)	147/905 (16.2%)	122/726 (16.8%)	25/179 (14.0%)	0.352
Ischemic HF Etiology					0.010
Ischemic	1197/2331 (51.4%)	460/910 (50.5%)	354/731 (48.4%)	106/179 (59.2%)	
Non-Ischemic	1134/2331 (48.6%)	450/910 (49.5%)	377/731 (51.6%)	73/179 (40.8%)	
NYHA class at random assignment					<.001
II	1477/2331 (63.4%)	570/910 (62.6%)	489/731 (66.9%)	81/179 (45.3%)	
III	831/2331 (35.6%)	328/910 (36.0%)	238/731 (32.6%)	90/179 (50.3%)	
IV	23/2331 (1.0%)	12/910 (1.3%)	4/731 (0.5%)	8/179 (4.5%)	
Systolic BP, mmHg	111.0 (100.0,126.0)	112.0 (100.5,126.5)	114.0 (102.0,128.0)	110.0 (100.0,126.0)	0.153
Resting HR, bpm	70.0 (63.0,79.0)	71.0 (63.0,80.0)	70.0 (63.0,79.0)	73.0 (66.0,81.0)	0.007
LVEF, %	24.7 (20.0,30.1)	24.4 (19.4,30.0)	24.8 (19.5,30.1)	23.2 (18.2,28.4)	0.051
Creatinine, mg/dL	1.2 (1.0,1.5)	1.2 (1.0,1.5)	1.2 (1.0,1.4)	1.3 (1.1,1.8)	<.001
Blood urea nitrogen, mg/dL	20.0 (15.0,28.0)	21.0 (15.0,28.0)	19.0 (14.0,26.0)	25.0 (18.0,35.3)	<.001
Hemoglobin, g/dL	13.5 (12.3,14.6)	13.3 (12.2,14.5)	13.3 (12.3,14.6)	13.2 (11.9,14.2)	0.019
NTproBNP (Baseline)	815.0 (340.5,1805)	857.0 (359.3,1955)	671.8 (311.6,1473)	2212 (976.0,4995)	<.001
Exercise duration, CPX test, min	9.6 (6.9,12.0)	9.5 (7.0,12.0)	10.0 (7.7,12.4)	7.4 (5.3,9.5)	<.001
Peak V02 in mL/kg/min CPX test at baseline	14.4 (11.5,17.7)	14.5 (11.6,17.4)	15.2 (12.2,18.0)	12.1 (9.8,14.5)	<.001
6-Minute walk distance, m	370.6 (298.7,435.0)	368.5 (297.2,432.8)	378.3 (313.9,442.0)	315.2 (259.1,388.6)	<.001
Loop diuretic dose, mg/day	40.0 (20.0,80.0)	40.0 (20.0,80.0)	40.0 (10.0,80.0)	60.0 (40.0,120.0)	<.001
Mitral Regurgitation, moderate or severe	256/2135 (12.0%)	98/840 (11.7%)	65/679 (9.6%)	33/161 (20.5%)	<.001
VE/VCO2 slope	32.6 (28.1,38.5)	32.4 (28.0,38.0)	31.3 (27.6,36.3)	36.9 (32.4,44.8)	<.001

Variable	Total Population (N=2331)	Substudy Population (N=912)	ST2 35ng/mL (N=731)	ST2 > 35ng/mL (N=179)	P-Value
KCCQ symptom stability score	50.0 (50.0,50.0)	50.0 (50.0,50.0)	50.0 (50.0,50.0)	50.0 (50.0,50.0)	0.323
Rest ECG ventricular conduction					<.001
Normal	979/2271 (43.1%)	390/891 (43.8%)	331/714 (46.4%)	59/177 (33.3%)	
LBBB	379/2271 (16.7%)	135/891 (15.2%)	116/714 (16.2%)	19/177 (10.7%)	
RBBB	85/2271 (3.7%)	30/891 (3.4%)	22/714 (3.1%)	8/177 (4.5%)	
IVCD	292/2271 (12.9%)	97/891 (10.9%)	74/714 (10.4%)	23/177 (13.0%)	
Paced	536/2271 (23.6%)	239/891 (26.8%)	171/714 (23.9%)	68/177 (38.4%)	
Region: US v Non-US					0.012
0	2068/2331 (88.7%)	851/910 (93.5%)	691/731 (94.5%)	160/179 (89.4%)	
1	263/2331 (11.3%)	59/910 (6.5%)	40/731 (5.5%)	19/179 (10.6%)	
BB Dose at baseline	25.0 (13.0,50.0)	25.0 (13.0,50.0)	37.5 (13.0,50.0)	25.0 (13.0,50.0)	0.228
BMI	29.9 (26.0,35.1)	30.4 (26.4,35.7)	30.9 (26.5,35.9)	29.2 (25.1,35.1)	0.018
Canadian Angina Class	200/2328 (8.6%)	92/909 (10.1%)	76/730 (10.4%)	16/179 (8.9%)	0.744
HF Hosp in prior 6 months	610/2311 (26.4%)	243/903 (26.9%)	196/725 (27.0%)	47/178 (26.4%)	0.865

Data are presented as medians (Q1, Q3) or percentages.

BP = blood pressure; BUN = blood urea nitrogen; CPX = cardiopulmonary exercise; HF = heart failure; LVEF; left ventricular ejection fraction; MI = myocardial infarction; NYHA = New York Heart Association; peak VO<sub>2</sub> = maximal oxygen consumption; NT-proBNP = amino-terminal pro-B-type natriuretic peptide.

**Table 2**

Multivariable Models for ST2 and Risk for Long Term Outcomes

	All-Cause Death/Hospitalization			CV Death/HF Hospitalization			All-Cause Death			CV Death			HF Hospitalization		
	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
ST2 univariable	1.48	1.32-1.66	<0.0001	2.14	1.84-2.49	<0.0001	2.33	1.93-2.81	<0.0001	2.53	2.04-3.14	<0.0001	2.00	1.69-2.36	<0.0001
<b>Demographics</b>															
+ age, sex, race	1.42	1.26-1.61	<0.0001	2.07	1.78-2.42	<0.0001	1.99	1.63-2.43	<0.0001	2.30	1.82-2.90	<0.0001	1.98	1.67-2.34	<0.0001
+ age, sex, race, NT-proBNP	1.17	1.02-1.34	0.0210	1.47	1.22-1.77	<0.0001	1.52	1.20-1.93	0.0006	1.68	1.26-2.24	0.0004	1.44	1.18-1.77	0.0005
<b>Final clinical model without CPX variables</b>															
+ age, sex, race	1.29	1.13-1.50	0.0003	1.68	1.40-2.02	<0.0001	1.74	1.40-2.16	<0.0001	1.98	1.50-2.60	<0.0001	1.52	1.25-1.86	<0.0001
+ age, sex, race, NT-proBNP	1.13	0.97-1.33	0.1213	1.37	1.11-1.69	0.0037	1.42	1.10-1.82	0.0067	1.60	1.15-2.21	0.0047	1.32	1.05-1.65	0.0174
<b>Final clinical model with CPX variables</b>															
+ age, sex, race	1.21	1.05-1.40	0.0072	1.50	1.24-1.82	<0.0001	1.47	1.18-1.85	0.0008	1.70	1.27-2.27	0.0004	1.35	1.10-1.66	0.0047
+ age, sex, race, NT-proBNP	1.08	0.92-1.27	0.3605	1.32	1.06-1.63	0.0129	1.26	0.97-1.63	0.0877	1.54	1.10-2.14	0.0114	1.22	0.97-1.54	0.0967

HR = Hazard ratio, CI = confidence interval, NT-proBNP = amino-terminal-pro-b-type natriuretic peptide, CPX = cardiopulmonary exercise test

**Table 3**

Added Value of ST2 to Final Clinical Model and NT-proBNP

C-Statistics	All-Cause Death/Hospitalization	CV Death/HF Hospitalization	All-Cause Death	CV Death	HF Hospitalization
Without CPX variables + age, race, NT-proBNP	0.641	0.759	0.751	0.778	0.762
Without CPX variables + age, race, NT-proBNP + ST2	0.644	0.768	0.758	0.789	0.771
<b>95% CI for Change in C-Statistic</b>	(0.0003, 0.0048)	(0.0084, 0.0155)	(0.0043, 0.0125)	(0.0055, 0.0145)	(0.0079, 0.0149)
<b>IDI</b>	0.0002	0.0065	0.0078	0.0123	0.0043
<b>95% CI for IDI</b>	(-0.044, 0.0027)	(-0.0293, 0.0181)	(-0.0282, 0.0597)	(-0.0059, 0.0264)	(-0.0056, 0.0123)
<b>NRI</b>	0.025	0.015	0.049	0.0472	0.0638
<b>95% CI for NRI</b>	(-0.1142, 0.187)	(-0.2123, 0.2468)	(-0.2222, 0.1492)	(-0.3260, 0.1812)	(-0.0985, 0.2763)

CI = confidence interval, CV = cardiovascular, IDI = integrated discrimination improvement, NRI = net reclassification improvement (without categories)