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Abstract

***Background*—**Soluble ST2 reflects activity of an IL-33 dependent cardioprotective signaling axis and is a diagnostic and prognostic marker in acute heart failure. The use of ST2 in chronic heart failure has not been well defined. Our objective was to determine whether plasma ST2 levels predict adverse outcomes in chronic heart failure in the context of current approaches.

***Methods and Results*—**We determined the association between ST2 level and risk of death or transplantation in a multi-center prospective cohort of 1,141 chronic heart failure outpatients. Adjusted Cox models, receiver operating characteristic (ROC) analyses, and risk reclassification metrics were used to assess the value of ST2 in predicting risk beyond currently used factors. After a median of 2.8 years, 267 patients (23%) died or underwent heart transplantation. Patients in the highest ST2 tertile (ST2>36.3ng/ml) had a markedly increased risk of adverse outcomes compared to the lowest tertile (ST2≤22.3ng/ml), with an unadjusted hazard ratio (HR) of 3.2 (95%CI:2.2-4.7;p<0.0001) that remained significant after multivariable adjustment (adjusted HR 1.9[95%CI:1.3-2.9];p=0.002). In ROC analyses, the area under the curve (AUC) for ST2 was 0.75 (95%CI:0.69-0.79), which was similar to NT-proBNP (AUC 0.77 [95%CI:0.72-0.81];p=0.24 versus ST2), but lower than the Seattle Heart Failure Model (SHFM; AUC 0.81 ([95%CI:0.77-0.85];p=0.014 versus ST2). Addition of ST2 and NT-proBNP to the SHFM reclassified 14.9% of patients into more appropriate risk categories (p=0.017).

***Conclusions*—**ST2 is a potent marker of risk in chronic heart failure and when used in combination with NT-proBNP offers moderate improvement in assessing prognosis beyond clinical risk scores.

Key Words: ST2, chronic heart failure, cardiomyopathy

Heart failure accounts for over one million hospitalizations and 60,000 deaths in the United States alone each year.[1] There is substantial variation in the severity and prognosis of heart failure, ranging from mild disease that is easily managed with oral neurohormonal blockade to advanced illness requiring therapy with mechanical support or heart transplantation.[2] Accurately assessing the risk of adverse outcomes is critical in order to advise patients and to guide the use of existing and emerging treatment strategies.

As heart failure progresses, clinical symptoms arise via interactions between cardiac dysfunction and maladaptive compensatory processes including vascular load, renal salt and water retention, neurohormonal activation, oxidative stress, and inflammation.[3] Circulating biomarkers that quantify activity of these biological pathways have thus been proposed as risk markers for gauging prognosis, and assessment of natriuretic peptide levels is now in widespread clinical use for this purpose. However, given the complexity of this syndrome it is unlikely that a single biomarker will be adequate, and additional measures that capture newly identified aspects of heart failure may improve risk stratification.[3]

The ST2 receptor is a member of the Toll-like/interleukin-1 (IL-1) receptor family. Research in animal models has shown that the cytokine IL-33 interacts with ST2 receptors on cardiac myocytes, comprising a cardioprotective stress-responsive signaling system.[4, 5] ST2 exists in both transmembrane and soluble forms, and soluble ST2 is a candidate biomarker in cardiovascular disease. In 813 subjects with acute myocardial infarction, ST2 levels at presentation independently predicted 30-day mortality[6, 7] and levels correlated with post-infarct remodeling.[8] In heart failure, assessment of ST2 levels during an episode of acute decompensation predicted increased mortality at 1 year.[9, 10] Studies of ST2 in chronic heart

failure have been less clear, in part due to small sample sizes,[11, 12] and in part due to first generation ST2 assays with limited sensitivity.[13]

The purpose of this study was to critically evaluate ST2 as a risk predictor in a large heart failure cohort using a new high-sensitivity assay[13] and to compare its performance to established risk predictors. We quantified soluble ST2 in 1,141 subjects from the Penn Heart Failure Study, a multi-center prospective cohort study of chronic heart failure patients representing a broad range of disease severity. We evaluated the strength and independence of the association between ST2 and transplant-free survival, the utility of ST2 in discriminating individual patient risk, and the added value of ST2 when used in combination with two established risk predictors: natriuretic peptide levels and the Seattle Heart Failure Model (SHFM) score.

Methods

Study Population

The Penn Heart Failure Study (PHFS) is a multi-center prospective cohort study of outpatients with primarily chronic systolic heart failure recruited from referral centers at the University of Pennsylvania (Philadelphia, PA), Case Western University (Cleveland, OH), and the University of Wisconsin (Madison, WI).[14, 15] The primary inclusion criterion is a clinical diagnosis of heart failure. Participants are excluded if they have a non-cardiac condition resulting in an expected mortality of less than 6 months as judged by the treating physician, or if they were unable or unwilling to provide informed consent.

At time of study entry, detailed clinical data were obtained using a standardized questionnaire administered to the patient and treating physician, with verification via medical records. Venous blood samples were obtained at enrollment, processed, and stored at -80^{o}C until

time of assay. Two-dimensional transthoracic echocardiography was performed in all patients at an ICAEL-accredited laboratory typically within 30 days of blood sampling with left ventricular ejection fraction (EF) visually estimated by a Level III-certified echocardiographer at each enrolling site.

Follow-up events including all-cause mortality and cardiac transplantation were prospectively ascertained every 6 months via direct patient contact and verified through death certificates, medical records, and contact with patients' family members by dedicated research personnel.

All participants provided written, informed consent, and the PHFS protocol was approved by participating Institutional Review Boards.

ST2 Assay

ST2 was measured from banked plasma samples via a highly sensitive sandwich monoclonal immunoassay (Presage™ ST2 assay, Critical Diagnostics, New York, New York). This platform offers improved accuracy in quantifying ST2 levels, particularly at lower concentrations. The antibodies used in the Presage™ assay were generated from a recombinant protein based upon the human cDNA clone for the complete soluble ST2 sequence.[13] The intra- and inter-assay coefficients of variation (CV) were less than 4.0% and 2.5%, respectively. The lower limit of detection of ST2 was 2 ng/ml and the upper limit was 200 ng/ml.

NT-proBNP Assay

Plasma NT-proBNP was measured by a standard electrochemiluminesence immunoassay (Elecsys proBNP, Roche Diagnostics, Indianapolis, Indiana), as previously described.[16] The

assay range was 20 to 5000 pg/ml. The intra- and interassay CV were 2.9 and 6.1%, respectively. NT-proBNP levels were measured from a banked plasma aliquot from the same baseline blood sample from which ST2 was quantitated.

Seattle Heart Failure Model

The Seattle Heart Failure Model (SHFM) is a multivariable risk prediction scoring system that has been validated in multiple heart failure study populations as a predictor of mortality.[17, 18] The version of the score used in this study was the SHFM-D which is based upon the following clinically assessed variables: age, gender, New York Heart Association (NYHA) class, ischemic etiology, left ventricular ejection fraction, medications (angiotensin converting enzyme inhibitor/angiotensin receptor blocker use, beta-blocker use, carvedilol use, statin use, furosemide equivalent daily dose, digoxin use), and laboratory values (serum sodium and creatinine). The derivation and validation of the SHFM-D has been previously described.[19]

Statistical Analyses

Associations between ST2 tertiles and relevant clinical variables were tested using ANOVA for symmetric continuous, Kruskal-Wallis tests for non-symmetric continuous, and χ^2 tests for categorical variables. Additionally, independent determinants of baseline ST2 levels were assessed using multivariable linear regression methods with the natural log transformed form of ST2 as the dependent variable. The inclusion of adjustment variables was defined using clinical judgment and statistical significance according to a stepwise model selection procedure based on Akaike information criteria (AIC). The effect of each determinant was derived from exponentiated regression coefficients (β). Cox models were used to determine the univariate

associations between ST2 tertiles and time to the combined endpoint of all-cause death or cardiac transplantation. Models for the continuous form of ST2 after $\log_2$ transformation were also constructed, in which the hazard ratio (HR) compared the risk between patient populations whose ST2 level differed by a multiplicative factor of 2. Multivariable models included covariates based upon statistical evidence for confounding and/or clinical judgment. Differences in ST2 association across groups defined by heart failure etiology were evaluated by including an interaction term between $\log_2$ST2 and an indicator of ischemic versus nonischemic cause of disease. In all Cox models, the baseline hazard function was stratified by NYHA classification (Class I, II, III, or IV) to account for different baseline risks of adverse clinical outcomes, given subjects entered the cohort at various disease stages. The proportional hazards assumption was verified using weighted residuals.[20]

To compare the association between ST2 and clinical outcomes with that of established risk predictors, models including ST2, the SHFM risk score, and NT-proBNP were constructed. The joint effects of ST2 and NT-proBNP were evaluated by dividing the cohort into 4 groups based upon median ST2 and NT-proBNP levels. To evaluate ST2 as discriminator of risk, two approaches were used. First, time-dependent receiver operating characteristic (ROC) curves were used to compare the ability of ST2, SHFM, and NT-proBNP to classify patients with regard to death or transplantation at 1 year.[21] Confidence intervals for the area under the ROC curve (AUC) were obtained from 1,000 bootstrapped samples, and AUCs were compared using Wald tests. Second, the incremental value of ST2 compared to the SHFM and NT-proBNP in predicting outcomes at 1 year was determined using net reclassification improvement (NRI).[22, 23] NRI is the difference in the number of patients moving up or down clinical risk groups, stratified according to whether or not they developed the outcomes during follow-up. Here, clinically

meaningful risk cutpoints of 0% to less than 10%, 10% to less than 20%, 20% to less than 50%, and ≥50% risk were defined *a priori*. Because some patients were censored before 1 year, the number of cases and controls at 1 year was estimated from the Kaplan-Meier survival estimator and confidence intervals were obtained via bootstrap estimation.[24] All statistical analyses were completed using R 2.9.0, including the survival, survivalROC, and pec packages.[25-28]

All authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

Results

Study Population

The distributions of clinical and laboratory variables across the entire cohort and according to ST2 tertile are displayed in Table 1. Across the entire cohort, the mean age was 56±14 years and the majority were male (67%). Nonischemic heart failure was more common than ischemic, and 87% of the population was classified by the treating cardiologist as having systolic heart failure. The mean ejection fraction was 32±17%. The median was 27.5 ng/ml (interquartile range 19.4-43.2 ng/ml). Participants in the highest ST2 tertile (ST2>36.3 ng/ml) were more likely to be older and male, with more advanced NYHA Class, and comorbid conditions such as hypertension and diabetes. In multivariable analyses, independent determinants of baseline ST2 levels were age, gender, race, NYHA Class, systolic blood pressure, ACE-inhibitor or angiotensin receptor blocker use, beta-blocker use, and NT-proBNP levels (Table 2).

Association between ST2 and Risk of All-cause Death or Cardiac Transplantation

Over a median follow-up of 2.8 years, there were 160 deaths and 107 cardiac transplantations. Higher ST2 levels were associated with a significantly increased risk of reaching this combined endpoint (Figure 1A). Participants in the highest ST2 tertile (ST2>36.3 ng/ml) had an unadjusted hazard ratio (HR) of 3.2 (95% CI, 2.2-4.7; p<0.0001) compared to the lowest tertile; and in multivariable models, this association remained robust (Table 3). Examining this relationship according to decile cutpoints revealed that this was predominantly a graded effect (Supplementary Figure 1). On a continuous scale, with each doubling of ST2, there was a 40-50% increased risk of death or cardiac transplantation in fully adjusted models.

Given the underlying biologic differences between ischemic and nonischemic heart failure, we explored whether the association between ST2 and risk of death or cardiac transplantation differed in these 2 groups. The risk associated with increasing levels of ST2 appeared more pronounced in the 784 patients with nonischemic heart failure (adjusted HR 1.7; 95% CI, 1.4-2.0; p<0.0001), compared to the 362 individuals with an ischemic cause of their heart failure (adjusted HR 1.3; 95% CI, 1.0-1.6; p=0.024), with a significant interaction p-value of 0.022 (adjusted for covariates in Table 3, Model 2).

Joint Effects of ST2 and NT-proBNP Assessment

There was only a moderate correlation between ST2 and NT-proBNP (R=0.41, p<0.0001), indicating that these two markers assess different aspects of the heart failure syndrome. To determine the potential utility of simultaneous ST2 and NT-proBNP assessment, we divided the cohort into 4 groups based upon a median ST2 level of 27.5 ng/ml and median NT-proBNP level of 566 pg/ml. As shown in Figure 1B, patients with either an elevated ST2 or NT-proBNP level had an increased risk compared to the reference group of subjects with low

levels of both markers (HR 1.8; 95% CI, 1.1-3.2; p=0.029 and HR 1.5; 95% CI, 0.9-2.6; p=0.11, respectively when adjusted for covariates in Table 3, Model 2). Patients with elevations in both ST2 and NT-proBNP had a markedly increased risk (adjusted HR 2.9; 95% CI, 1.8-4.6; p<0.0001), indicating that assessment of both ST2 and NT-proBNP is more effective at identifying a high risk subgroup than individual assessment of either biomarker.

Performance Characteristics of ST2 as a Discriminator of Individual Patient Risk

We used receiver operating characteristic (ROC) curve analyses to evaluate the ability of ST2 to classify individual patients according to those who did or did not experience death or transplantation at 1 year (Figure 2A). An ST2 level of 36.3 ng/ml was associated with a sensitivity 0.64 and specificity of 0.71, and a level of 34.9 ng/ml provided the maximum combination of sensitivity and specificity with a Youden index of 0.35 (Supplementary Table 1). The area under the curve (AUC) for ST2 was 0.75 (95% CI, 0.69-0.79), indicating a substantial capacity to discriminate high and low risk patients. Of note, this AUC was not statistically different (p=0.24) from that of NT-proBNP (AUC = 0.77; 95% CI, 0.72-0.81). When these two markers were used in combination, there was a significant improvement in the AUC to 0.80 (95% CI, 0.76-0.84) compared to either biomarker alone (p=0.0007 vs. ST2 alone and p=0.049 vs. NT-proBNP alone). The SHFM score, which summarizes a variety of clinical risk factors including physician-assessed NYHA Class, specific medication use and dosage, laboratory values, and ejection fraction by echocardiography, had an AUC of 0.81 (95% CI, 0.77-0.85), which was greater than ST2 alone (p=0.014), but similar to the 2 biomarkers together (p=0.55). Combining the SHFM with ST2 and NT-proBNP did not substantially improve the AUC compared to biomarkers or SHFM alone (Figure 2B).

Given the relative insensitivity in detecting potentially clinically important risk differences using AUC methods, and the growing role of risk reclassification as an important metric in assessing biomarker performance,[22, 23, 29, 30] the net reclassification improvement (NRI) was also quantified.[22, 23] We assessed whether adding ST2 alone and in combination with NT-proBNP to the SHFM score improved classification of patient risk at 1 year. We selected clinically meaningful heart failure risk categories of 0% to less than 10%, 10% to less than 20%, 20% to less than 50%, and ≥50% risk.

After classifying patients into risk groups using the SHFM (Table 4), addition of ST2 and NT-proBNP reclassified 14.9% of patients into more appropriate risk groups by assigning 35.3 of 148.6 cases as higher risk (p=0.016) and 103.9 of 976.4 controls as lesser risk (p=0.30). After classifying patients into risk groups using the combination of SHFM and NT-proBNP (Table 5), ST2 did not provide additive information (NRI 6.4%, 95% CI -1.9%-18.7%, p=0.23). Altogether, these findings indicate that ST2 levels, in combination with NT-proBNP, provide a moderate improvement beyond conventional prognostic factors in the upward- and downward-classification of patients into clinically meaningful risk categories.

Discussion

We report the largest study of the novel biomarker ST2 performed to date. In an ambulatory population of 1,141 patients with chronic heart failure, we found that ST2 is strongly associated with measures of worse heart failure severity, and patients with elevated levels of circulating ST2 had a markedly increased risk of death or heart transplantation. When used to assess individual patient risk, ST2 alone performed equivalently to the established biomarker NT-proBNP but was not significantly better than the SHFM alone. Adding ST2 and NT-

proBNP levels to the SHFM score improved risk discrimination by reclassifying 14.9% of patients into more appropriate categories. These findings demonstrate that ST2 is a potent indicator of prognosis in chronic heart failure, and offers a moderate improvement in risk stratification when used in combination with conventional markers.

ST2 is an IL-1 receptor family member expressed in cardiomyocytes, fibroblasts, and vascular endothelial cells.[31] ST2 is part of a cardioprotective signaling system comprised of paracrine interactions between IL-33 produced by cardiac fibroblasts and transmembrane ST2 receptors on cardiac myocytes.[4, 5] Mice treated with exogenous IL-33 demonstrate reduced hypertrophy, and transgenic deletion of ST2 abolishes this salutary effect, resulting in severe myocardial fibrosis and hypertrophy.[5] In response to inflammation and cardiac stress, IL-33/ST-2 signaling becomes activated and the soluble form of ST2 is released into the circulation. The soluble form of ST2 acts a decoy receptor, sequestering and inhibiting IL-33, potentially explaining why we and others have observed that higher circulating levels reflect increased cardiac risk.[4]

Our results extend previously published reports demonstrating that elevated ST2 levels are associated with worse outcomes in patients with cardiovascular disease. These studies have focused principally on ST2 as a risk predictor in the setting of acute myocardial infarction [6-8] and acutely decompensated heart failure,[9, 32, 33] with limited data on ST2 in chronic heart failure.[11, 12] A previous report in 161 patients with NYHA Class III-IV nonischemic heart failure suggested that serial change in ST2, but not baseline levels, was associated with an increased risk of death or transplantation.[12] In contrast, we found a robust, independent association between a single baseline measure of ST2 and risk of adverse outcomes. These differences are probably due to our use of a more sensitive ST2 assay, larger sample size, and assessment of ST2 in a broader

spectrum of heart failure patients. Interestingly, we also found that the relationship between ST2 and outcomes was stronger in nonischemic compared to ischemic heart failure. Research in model systems is required to rigorously test the hypothesis that IL-33/ST2 signaling plays a more prominent role in nonischemic heart failure.

Our study raises important points regarding the utility of biomarkers in the management of chronic heart failure. Criteria for evaluating new biomarkers include ease of measurability, added value to existing tests, mechanistic insight into the pathogenesis of disease, and improved clinical management of patients.[34] Although ST2 is easily and reproducibly measured and provides insight into the physiologic response to myocyte injury, its added value to established risk predictors is primarily a modest improvement in risk discrimination. Indeed, it is noteworthy that the SHFM score alone, without the use of any biomarker, is a potent discriminator of risk (Figure 2B). Thus ST2 and other heart failure biomarkers may have their greatest clinical utility in settings where the components of the SHFM risk score, such as physician assessed NYHA class and left ventricular ejection fraction, are unavailable or when these data are available but the treating physician remains uncertain regarding prognosis. Biomarkers may also provide additional mechanistic insight into the biologic alterations that occur with the complex syndrome of heart failure.

We acknowledge several limitations. By recruiting patients from referral centers, we studied a cohort that spans the full spectrum of heart failure severity; however, risk estimates may differ in populations with less severe disease or in patients with different demographics, such as the elderly. We were unable to perform serial assessments of ST2, which may be important given that serial changes in ST2 levels have been associated with adverse outcomes.

We were unable to measure levels of the ligand IL-33 or CRP, which may offer additional mechanistic insight into our observed associations.

In summary, we found that ST2 is a potent marker of risk in chronic heart failure and in combination with NT-proBNP, offers modest improvement in assessing prognosis beyond established clinical risk scores. Assessment of ST2 may aid in risk stratification to counsel heart failure patients and to focus more aggressive treatment strategies on those at highest risk. Our findings suggest that the utility of ST2 may be greatest in defining risk when physician-assessed clinical risk scores are unavailable and in providing mechanistic insight into the underlying pathophysiologic derangements of heart failure.

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Table 1. Baseline Cohort Characteristics According to ST2 Tertile*

	Entire Cohort	ST2 Tertile			
		First Tertile	Second Tertile	Third Tertile	p value †
		ST2 ≤ 22.3 ng/ml	22.3 < ST2 ≤ 36.3 ng/ml	ST2 > 36.3 ng/ml	
	n = 1141	*n* = 384	*n* = 378	*n* = 379	
Age, years	56.3 (14)	52.3 (13)	57.2 (14)	59.5 (14)	<0.0001
Male, *n* (%)	763 (67%)	202 (53%)	264 (70%)	297 (78%)	<0.0001
Race, *n* (%)					<0.0001
Caucasian	853 (75%)	248 (65%)	306 (81%)	299 (79%)	
African-American	236 (21%)	113 (29%)	63 (17%)	60 (16%)	
Other	42 (4%)	20 (5%)	7 (2%)	15 (4%)	
NYHA functional classification, *n* (%)					<0.0001
I	164 (14%)	72 (19%)	66 (17%)	26 (7%)	
II	523 (46%)	210 (55%)	177 (47%)	136 (36%)	
III	340 (30%)	90 (23%)	114 (30%)	136 (36%)	
IV	113 (10%)	11 (3%)	21 (6%)	81 (21%)	
Cardiomyopathy etiology					
Ischemic etiology, *n* (%)	359 (31%)	83 (22%)	112 (30%)	164 (43%)	<0.0001
Systolic etiology, *n* (%)	994 (87%)	332 (86%)	337 (89%)	325 (86%)	0.40
Tobacco use, *n* (%)					0.007
Never	433 (38%)	163 (42%)	148 (39%)	122 (32%)	
Current	92 (8%)	38 (10%)	25 (7%)	29 (8%)	
Former	612 (54%)	181 (47%)	204 (54%)	227 (60%)	
History of hypertension, *n* (%)	632 (55%)	197 (51%)	206 (54%)	229 (60%)	0.037
History of diabetes, *n* (%)	325 (28%)	81 (21%)	108 (29%)	136 (36%)	<0.0001
Body mass index, kg/m^2	29.7 (7.1)	30.1 (7.5)	30.0 (7.0)	29.1 (6.5)	0.040
Systolic blood pressure, mmHg	114 (20)	116 (19)	116 (21)	111 (19)	0.002
Creatinine, mg/dL	1.33 (0.86)	1.16 (0.71)	1.33 (0.99)	1.51 (0.81)	<0.0001
Ejection fraction, %	32.2 (17)	33.9 (16)	32.4 (16)	30.3 (17)	0.003
Biventricular pacemaker, *n* (%)	304 (27%)	71 (18%)	105 (28%)	128 (34%)	<0.0001
Internal cardiac defibrillator, *n* (%)	497 (44%)	136 (35%)	163 (43%)	198 (52%)	<0.0001
Medication use					
ACE inhibitors/ARBs, *n* (%)	978 (86%)	348 (91%)	333 (88%)	297 (78%)	<0.0001
Aldosterone antagonists, *n* (%)	371 (33%)	123 (32%)	120 (32%)	128 (34%)	0.81
Beta blockers, *n* (%)	982 (86%)	348 (91%)	330 (87%)	304 (80%)	0.0001
Digoxin, *n* (%)	487 (43%)	134 (35%)	164 (43%)	189 (50%)	0.0002
Diuretics, *n* (%)	841 (74%)	263 (68%)	270 (71%)	308 (81%)	0.0002
NT-proBNP, pg/ml, median (IQR)	567 (172, 1670)	285 (105, 826)	444 (160, 1230)	1460 (503, 3390)	<0.0001
Seattle Heart Failure Model	0.25 (1.0)	-0.23 (0.73)	0.18 (0.90)	0.82 (1.0)	<0.0001

*Data presented as mean (standard deviation), unless otherwise noted as *n* (%) or median (IQR; inter-quartile range); †Based on ANOVA for symmetric continuous variables; Kruskal-Wallis test for non-symmetric continuous variables; χ^2 test for categorical variables;

Table 2. Independent Determinants of Baseline ST2 Levels

	Difference in ST2*	95% CI	p value
Demographic Characteristics			
Age (10 year difference)	+3.0%	(+0.2%, +5.8%)	0.036
Male (vs female)	+25.4%	(+16.3%, +35.1%)	<0.0001
African American (vs Caucasian)	-11.6%	(-19.3%, -3.1%)	0.008
Medical History and Risk Factors			
History of hypertension (vs none)	+8.0%	(-0.1%, +16.8%)	0.052
History of diabetes (vs none)	+6.0%	(-2.2%, +14.9%)	0.16
Heart Failure Characteristics			
NYHA functional classification			
II (vs I)	-3.6%	(-13.5%, +7.6%)	0.52
III (vs I)	+12.5%	(-0.5%, +27.2%)	0.059
IV (vs I)	+57.4%	(+34.0%, +84.8%)	<0.001
Medication Use			
ACE-inhibitor/ARB use (vs none)	-11.8%	(-20.7%, -1.8%)	0.022
Beta-blocker (vs none)	-19.3%	(-27.4%, -10.2%)	<0.0001
Aldosterone Antagonist (vs none)	-6.1%	(-12.9%, +1.3%)	0.11
Clinical Measures			
Systolic blood pressure (10 mmHg difference)	-2.6%	(-4.4%, -0.7%)	0.008
Creatinine (0.5 mg/dl difference)	+1.9%	(-0.4%, +4.2%)	0.11
NT-proBNP (multiplicative difference of 2)	+7.9%	(+5.8%, +10.0%)	<0.0001

*This column denotes the exponentiated β coefficient from a multivariable linear regression model for natural-log transformed ST2, and represents the percent difference in ST2 between each group for categorical or continuous variables.

Table 3: Association between ST2 and Risk of Death or Cardiac Transplantation

	ST2 Tertile 3 versus 1 HR (95% CI)	p value	$\log_2$(ST2) HR (95% CI)	p value
Unadjusted	3.2 (2.2, 4.7)	p<0.0001	1.7 (1.5, 1.9)	p<0.0001
Model 1	3.0 (2.1, 4.4)	p<0.0001	1.7 (1.5, 1.9)	p<0.0001
Model 2	2.5 (1.7, 3.7)	p<0.0001	1.5 (1.3, 1.7)	p<0.0001
Model 3	2.2 (1.4, 3.2)	p=0.0003	1.4 (1.2, 1.7)	p<0.0001
Model 4	1.9 (1.3, 2.9)	p=0.002	1.4 (1.2, 1.6)	p<0.0001

Model 1: Adjusted for age, gender, and race
Model 2: Adjusted for Model 1 and cardiomyopathy etiology (ischemic versus nonischemic), tobacco use, body mass index, systolic blood pressure, creatinine, ejection fraction, biventricular pacemaker, cardioverter-defibrillator, ACE inhibitor/angiotensin receptor blocker, aldosterone antagonist, beta blocker therapy, and clinical site
Model 3: Adjusted for Model 2 and NT-proBNP
Model 4: Adjusted for Seattle Heart Failure Model and NT-proBNP

Table 4: Reclassification of 1-Year Risk with Addition of ST2 and NT-proBNP to Seattle Heart Failure Model (SHFM) Score

SHFM	SHFM plus ST2 and NT-proBNP*				
	0%-<10%	≥10%-<20%	≥20%-<50%	≥50%-100%	Total
0%-<10%					
All subjects	596	45	5	0	646
Case subjects†	17.2	7.0	1.0		25.3
Control subjects†	578.8	38.0	4.0		620.7
Observed risk (%)‡	2.9	15.6	20.0		3.9
≥10%-<20%					
All subjects	67	149	49	0	265
Case subjects†	2.0	18.2	17.3		37.4
Control subjects†	65.0	130.8	31.7		227.6
Observed risk (%)‡	3.0	12.2	35.3		14.1
≥20%-<50%					
All subjects	2	43	113	21	179
Case subjects†	1.0	11.1	40.1	10.0	62.1
Control subjects†	1.0	31.9	72.9	11.0	116.9
Observed risk (%)‡	50.0	25.7	35.4	47.6	34.7
≥50%-100%					
All subjects	0	0	8	27	35
Case subjects†			2.0	21.0	23.2
Control subjects†			6.0	6.0	11.8
Observed risk (%)‡			25.0	77.8	66.3
Total					
All subjects	665	237	175	48	1125
Case subjects†	20.3	36.4	60.5	31.0	148.6
Control subjects†	644.7	200.6	114.5	17.0	976.4
Observed risk (%)‡	3.1	15.4	34.5	64.6	13.2

*1,125 had an assessment of NT-proBNP, ST2, and the SHFM
† Estimated from 1-year Kaplan-Meier risk estimates
‡ Kaplan-Meier risk estimates at 1 year

NRI: 14.9%, 95% CI: (1.8%, 25.9%), p = 0.017
NRI (case subjects): 12.9%, 95% CI: (1.2%, 21.8%), p = 0.016
NRI (control subjects): 2.0%, 95% CI: (-1.3%, 6.4%), p = 0.30

Table 5: Reclassification of 1-Year Risk with Addition of ST2 to NT-proBNP and Seattle Heart Failure Model (SHFM) Score

SHFM plus NT-proBNP	SHFM plus ST2 and NT-proBNP* 0%-<10%	≥10%-<20%	≥20%-<50%	≥50%-100%	Total
0%-<10%					
All subjects	613	20	0	0	633
Case subjects†	19.2	3.0			22.2
Control subjects†	593.8	17.0			610.8
Observed risk (%)‡	3.1	15.0			3.5
≥10%-<20%					
All subjects	49	183	27	0	259
Case subjects†	0.0	23.1	6.2		29.3
Control subjects†	49.0	159.9	20.8		229.7
Observed risk (%)‡	0.0	12.6	22.8		11.3
≥20%-<50%					
All subjects	3	34	143	17	197
Case subjects†	1.0	10.4	52.2	9.0	72.4
Control subjects†	2.0	23.6	90.8	8.0	124.6
Observed risk (%)‡	33.3	30.7	36.5	52.9	36.8
≥50%-100%					
All subjects	0	0	5	31	36
Case subjects†			2.0	22.0	24.3
Control subjects†			3.0	9.0	11.7
Observed risk (%)‡			40.0	71.0	67.4
Total					
All subjects	665	237	175	48	1125
Case subjects†	20.3	36.4	60.5	31.0	148.6
Control subjects†	644.7	200.6	114.5	17.0	976.4
Observed risk (%)‡	3.1	15.4	34.5	64.6	13.2

*1,125 had an assessment of NT-proBNP, ST2, and the SHFM
† Estimated from 1-year Kaplan-Meier risk estimates
‡ Kaplan-Meier risk estimates at 1 year

NRI: 6.4%, 95% CI: (-1.9%, 18.7%), p = 0.23
NRI (case subjects): 3.2%, 95% CI: (-4.2%, 12.7%), p = 0.48
NRI (control subjects): 3.3%, 95% CI: (0.5%, 7.4%), p = 0.070

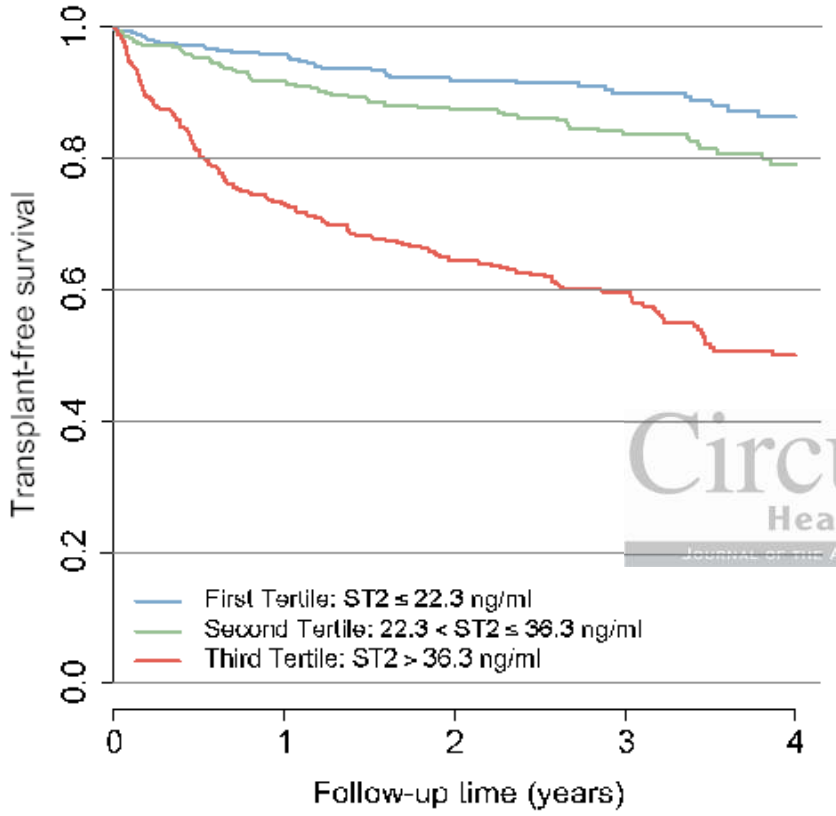
Figure Legends:

Figure 1. Transplant-Free Survival According to ST2 and NT-proBNP Levels

Kaplan-Meier plots illustrating the incidence of all-cause death or cardiac transplantation among Penn Heart Failure Study participants according to ST2 levels (A) and the joint assessment of ST2 and NT-proBNP (B). ($p<0.0001$ by log rank test for each panel)

Figure 2. Receiver Operating Characteristic Curves for ST2, NT-proBNP, and the SHFM for Transplant-Free Survival at 1 Year. Receiver operating characteristic (ROC) curves and the corresponding areas under the curve (AUC) for (A) biomarkers alone and (B) biomarkers and the SHFM score.

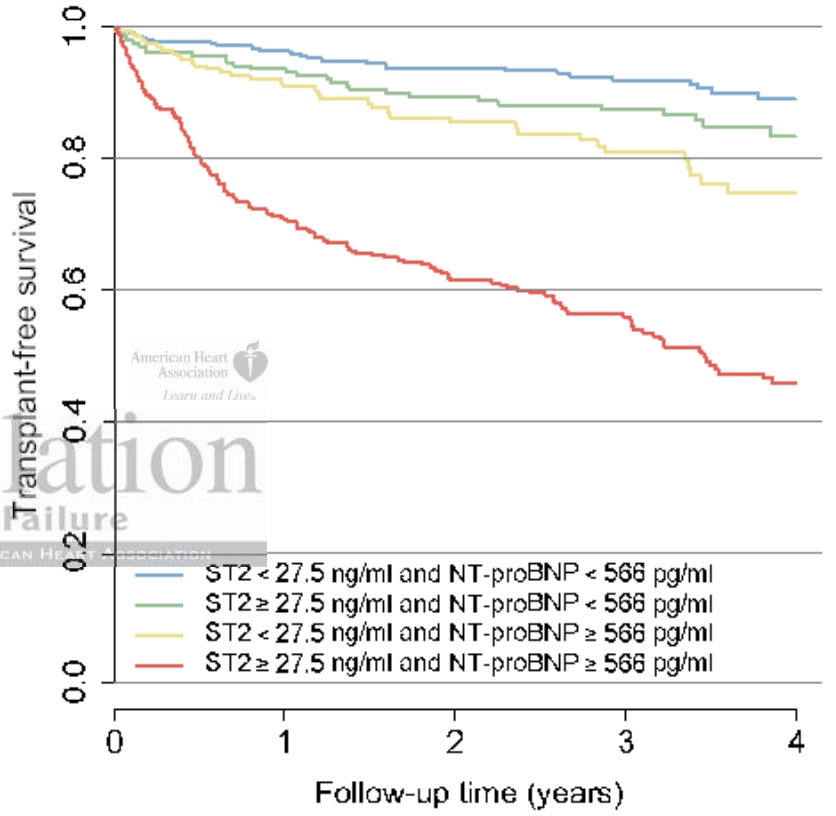
A.



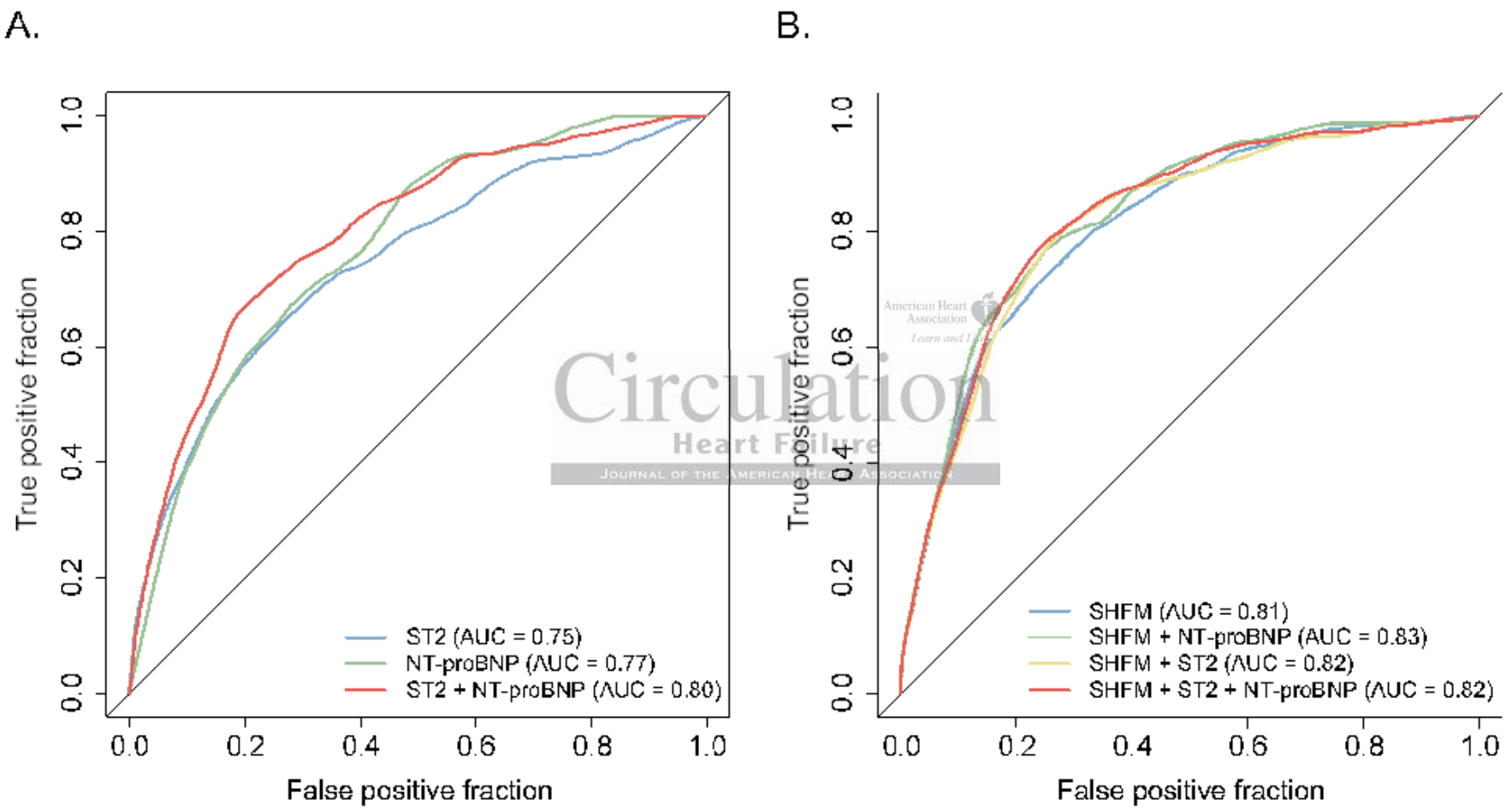
Number at risk

Year	0	1	2	3	4
	384	316	228	170	71
	378	316	248	187	74
	379	253	195	144	54

B.

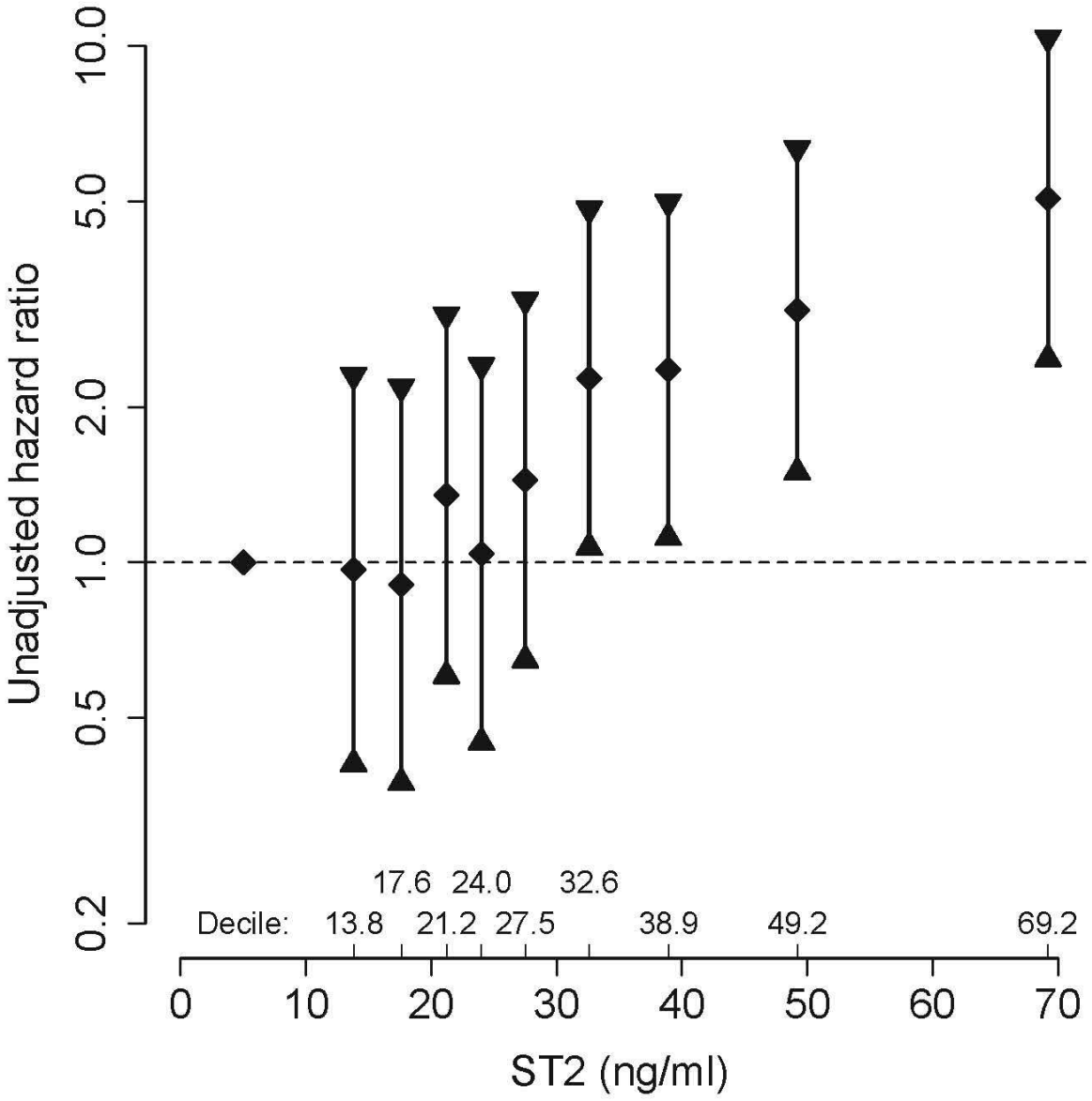


	0	1	2	3	4
	354	302	228	176	68
	207	181	154	120	44
	204	159	107	76	38
	360	229	171	122	49



SUPPLEMENTAL MATERIAL

Supplementary Figure 1: Hazard Ratios for All-Cause Death or Cardiac Transplantation According to ST2 Deciles



Supplementary Table 1: Sensitivity and specificity for ST2 cut-points

ST2 cut-point (ng/ml)	Sensitivity	Specificity
Observed tertiles		
22.3	0.88	0.37
36.3	0.64	0.71
Observed deciles		
13.8	0.96	0.11
17.6	0.93	0.22
21.2	0.90	0.33
24.0	0.83	0.43
27.5	0.77	0.54
32.6	0.71	0.64
38.9	0.61	0.74
49.2	0.48	0.84
69.2	0.29	0.93
Optimal cut-point*		
34.9	0.67	0.68

*Optimal cut-point: 34.9 ng/ml as defined by a Youden index of 0.35.