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Published in:
JACC. Heart failure

DOI:
[10.1016/j.jchf.2015.07.015](https://doi.org/10.1016/j.jchf.2015.07.015)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Tang, W. H. W., Wu, Y., Grodin, J. L., Hsu, A. P., Hernandez, A. F., Butler, J., Metra, M., Voors, A. A., Felker, G. M., Troughton, R. W., Mills, R. M., McMurray, J. J., Armstrong, P. W., O'Connor, C. M., & Starling, R. C. (2016). Prognostic Value of Baseline and Changes in Circulating Soluble ST2 Levels and the Effects of Nesiritide in Acute Decompensated Heart Failure. *JACC. Heart failure*, 4(1), 68-77. <https://doi.org/10.1016/j.jchf.2015.07.015>

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Prognostic Value of Baseline and Changes in Circulating Soluble ST2 Levels and the Effects of Nesiritide in Acute Decompensated Heart Failure

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ABSTRACT

OBJECTIVES The study sought to investigate the association between soluble growth stimulation expressed gene 2 (sST2) level and adverse outcomes in acute heart failure (HF).

BACKGROUND Several studies have demonstrated the prognostic utility of sST2 levels in HF.

METHODS sST2 levels were measured in sequential baseline and follow-up (48 to 72 h and 30 days) plasma samples from 858 acute HF subjects enrolled in the ASCEND-HF (Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure) trial biomarker substudy and were related to in-hospital and post-discharge clinical outcomes.

RESULTS Higher sST2 levels were associated with increased death risk at 180 days (baseline hazard ratio [HR]: 2.21; follow-up HR: 2.64; both $p < 0.001$). These results were not independent of covariates and aminoterminal pro-B-type natriuretic peptide for baseline sST2 (HR: 1.29, $p = 0.243$), but were borderline significant for follow-up sST2 (HR: 1.61, $p = 0.051$). Subjects with persistently high (>60 ng/ml) sST2 levels at follow-up had higher 180-day death rates than those with lower follow-up sST2 levels (adjusted HR: 2.91, $p = 0.004$). Neither baseline nor follow-up sST2 levels were associated with dyspnea improvement. Changes in sST2 from baseline were less in the nesiritide versus placebo group at follow-up, but were similar at 30 days.

CONCLUSIONS Elevated levels of sST2 were associated with an increased risk of adverse clinical events in acute HF, but prognostic value of baseline sST2 diminished after adjusting for clinical covariates and aminoterminal pro-B-type natriuretic peptide. In those with elevated baseline sST2 levels, persistently elevated sST2 levels at follow-up were associated with increased mortality risk. In addition, nesiritide did not demonstrate an incremental impact on sST2 levels over standard therapy. (J Am Coll Cardiol HF 2016;4:68-77) © 2016 by the American College of Cardiology Foundation.

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Growth stimulation expressed gene 2 (ST2) is a transmembrane protein and a member of the Toll-interleukin 1 receptor superfamily (1,2). ST2 binds interleukin-33 in response to cardiac disease or injury and elicits a cardioprotective effect by mitigating the maladaptive responses of the myocardium to overload states (3,4). A truncated soluble form of ST2 (soluble ST2 [sST2]) competes with the membrane-bound form in this interleukin-33 binding. Elevated levels of sST2 signal the presence and severity of adverse cardiac remodeling and tissue fibrosis, which may occur in response to an acute coronary syndrome event or worsening heart failure (HF) (3,5). Higher levels of sST2 are associated with more severe clinical symptoms and with other objective measures of HF severity, such as higher C-reactive protein, higher natriuretic peptide levels, lower left ventricular ejection fraction, and higher diastolic filling pressures (6-12). Elevated circulating sST2 levels have been associated with an increased risk for mortality and sudden cardiac death in outpatients with HF (9,13-15), as well as in acute HF (16). However, most studies have only measured sST2 at a single timepoint (predominantly at baseline) and only described the relationship with long-term all-cause mortality.

In this post-hoc study utilizing blood specimens collected serially in the ASCEND-HF (Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure) trial, we examined the relationship between baseline and serial levels of sST2 and dyspnea status, hospitalization (at 30 days), and death (at 180 days). We also examined the effect of nesiritide therapy on sST2 levels, hypothesizing that the vasodilatory effects of nesiritide may relieve volume overload more effectively than a placebo, thereby potentially achieving greater reduction in sST2 levels.

METHODS

STUDY POPULATION. Details of the ASCEND-HF Trial (NCT00475852) have been described elsewhere (17). Briefly, this was a multinational, multicenter,

prospective randomized controlled trial of 7,141 subjects presenting with signs and symptoms of acute decompensated HF comparing nesiritide (a recombinant B-type natriuretic peptide with vasodilatory properties) to placebo added to standard care. In our study cohort, 858 subjects (12% of the total population) consented to participate in the biomarker substudy. A large majority of subjects in the biomarker substudy were recruited from North American sites (n = 824). Compared to the rest of the North American study cohort (n = 2,419), there were no differences in race (p = 0.422), heart rate (p = 0.157), atrial fibrillation (p = 0.124), blood urea nitrogen (p = 0.384), creatinine (p = 0.499), time to randomization (p = 0.051), or beta-blockers (p = 0.073). Nevertheless, age (66.6 ± 14.9 vs. 64.5 ± 15.4 years, p = 0.001) and left ventricular ejection fraction (31.6 ± 15 vs. 30.4 ± 15, p = 0.035) were significantly different.

STUDY DESIGN. The intent of the biomarker substudy was to collect venous blood samples at randomization (“baseline”), 48 to 72 h following randomization, and at the 30-day follow-up visit. Blood samples were collected in ethylenediaminetetraacetic acid-plasma, immediately centrifuged at the study sites, and stored at -80°C for subsequent analysis at a central core laboratory. Aminoterminal pro-B-type natriuretic peptide (NT-proBNP) levels were determined by the VITROS NT-proBNP Assay (Ortho-Clinical Diagnostics, Raritan, New Jersey).

SOLUBLE ST2 ASSAY. Plasma sST2 levels were measured by the Presage ST2 Assay (Critical Diagnostics, San Diego, California) at a College of American Pathologists/Clinical Laboratory Improvements Amendments-approved core laboratory independent of the sponsors. This is a quantitative sandwich enzyme-linked immunosorbent assay using a mouse monoclonal antihuman sST2 capture antibody on microtiter plate wells and a second biotinylated mouse monoclonal antihuman sST2 tracer antibody with a measuring range of 3.1 to 200 ng/ml

ABBREVIATIONS AND ACRONYMS

CI = confidence interval
HF = heart failure
HR = hazard ratio
IQR = interquartile range
NT-proBNP = aminoterminal pro-B-type natriuretic peptide
OR = odds ratio
sST2 = soluble growth stimulation expressed gene 2

has received research grant support from Johnson & Johnson, Roche Diagnostics, Critical Diagnostics, and BG Medicine (significant); and has served as a consultant for Roche Diagnostics and Singulex. Dr. Troughton has served as a consultant for and received advisory board funding from St. Jude Medical (modest) and Roche Diagnostics. Dr. Mills is an employee of Janssen Research and Development, LLC (formerly Johnson & Johnson). Dr. McMurray has received research grant support from Johnson & Johnson (significant). Dr. Armstrong has received research grant support from Johnson & Johnson and Ortho Biotech (significant). Dr. O'Connor has received research grant support from Johnson & Johnson (significant), BG Medicine, Critical Diagnostics, and Roche Diagnostics; and has served as a consultant for Cardioentis. Dr. Starling has received research support from Johnson & Johnson (modest); consultant/advisory board for Johnson & Johnson (modest). All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. John R. Teerlink, MD, served as the Guest Editor for this paper.

Manuscript received October 8, 2014; revised manuscript received July 17, 2015, accepted July 29, 2015.

and a coefficient of variation <5%; the limit of detection is at 1.8 ng/ml, and the limit of quantification at 2.4 ng/ml.

CLINICAL ENDPOINTS. The following adjudicated endpoints were analyzed: 30-day death, 180-day death, 30-day HF hospitalization or death, and the composite of persistent or worsening HF or death from any cause. We also used the ASCEND-HF trial's copri-mary dyspnea endpoint (moderately or markedly improved relative to the time of randomization measured by a 7-point Likert-type scale at 6 or 24 h) to assess the association between sST2 levels and symp-tom relief.

STATISTICAL ANALYSES. Clinical characteristics are presented as a percentage (%) for categorical vari-ables, mean \pm SD for normally distributed contin-uous variables, and median (interquartile range [IQR]) for non-normally distributed continuous vari-ables. The Cochran-Armitage test was used to test for trend in baseline characteristics across increasing tertiles of sST2 at baseline. Survival curves are estimated for each group, considered separately, using the Kaplan-Meier method and compared stati-stically using the log-rank test. The association between sST2 and outcomes was performed using both univariate and multivariate logistic regression analysis (for 30-day outcomes) or Cox proportional hazards analysis (for 180-day mortality). Logistic regression was used in the analyses of dyspnea improvement. sST2 and NT-proBNP were both log transformed, and odds ratios (ORs) and hazard ra-tios (HRs) were analyzed using increments of sST2/NT-proBNP per log increase in sST2. For the multi-variate analysis, we adjusted the covariates identi-fied from the overall ASCEND-HF study population to be prognostically relevant (the ASCEND-HF risk model) (Online Table 1). We used the robust covariance matrix estimates to adjust the variance-covariance matrix of both logistic regression and Cox models to correct for correlated responses from cluster (multicenter) samples (18). To assess if the addition of sST2 to the ASCEND-HF risk model with NT-proBNP improves outcome prediction, we used the category-free net reclassification index by the Pencina method (19,20). Levels of sST2 at all time points and changes in sST2 from baseline were compared between subjects receiving nesiritide and placebo using the Wilcoxon rank sum test or Stu-dent *t* test. All statistical analyses were performed using Stata 13.1 software (StataCorp LP, College Station, Texas) and R 3.1.0 (Vienna, Austria). A 2-sided *p* value of <0.05 was considered statistically significant.

RESULTS

PATIENT CHARACTERISTICS. Baseline characteris-tics of the study population are illustrated in Table 1. The median time between presenting to the hospital and randomization (baseline) was 16 h. In our study cohort, median sST2 levels were 71.2 (IQR: 48.2 to 111.1) ng/ml at baseline, decreasing to 46.9 (IQR: 32.4 to 70.3) ng/ml at 48 to 72 h and 39.5 (IQR: 27.8 to 63.8) ng/ml at 30 days. In other words, 89% (763 of 858) of patients had sST2 levels above the diagnostic cutoff value of 35 ng/ml for chronic HF. Subjects with impaired or preserved left ventricular ejection frac-tion had similar levels of baseline sST2 (72.4 [IQR: 49.2 to 116.0] ng/ml vs. 68.9 [IQR: 45.1 to 108.3] ng/ml; *p* = 0.178, respectively).

BASELINE sST2 LEVELS AND PROGNOSIS. There were 24 (2.8%) deaths and 77 (9.2%) HF rehospitali-zations by 30 days, and 97 (11.4%) deaths by 180 days. Higher baseline sST2 level was associated with a higher risk of death at 30 days (OR: 2.33; 95% confi-dence interval [CI]: 1.05 to 5.19; *p* = 0.038) and at 180 days (HR: 2.21; 95% CI: 1.56 to 3.13; *p* < 0.001), as well as death/worsening HF before discharge (OR: 2.23; 95% CI: 1.28 to 3.90; *p* = 0.005) (Table 2). Figure 1A shows that increasing quartiles of baseline sST2 was associated with greater 180-day mortality risk by Kaplan-Meier estimates. In contrast, symp-tomatic relief at 6 h and at 24 h was not associated with higher levels of baseline sST2 (*p* > 0.29, data not shown). After adjusting for other risk covariates in the ASCEND-HF risk model, only 180-day mortality risk was associated with higher levels of baseline sST2 (adjusted HR: 1.79; 95% CI: 1.22 to 2.62; *p* = 0.003) (Table 2). However, further adjustment with the ASCEND-HF risk model plus baseline NT-proBNP levels demonstrated that the prognostic value of baseline sST2 was no longer significant (Table 2, as dichotomous variables in Online Table 2); this was true despite the fact that adding baseline sST2 to the ASCEND-HF risk model, plus baseline NT-proBNP, correctly reclassified 10.76% of subjects for the 180-day death endpoint (with 8.64% events correctly classified and 2.12% nonevents correctly classified) (Online Table 3A). Interestingly, interaction testing between baseline sST2 and baseline NT-proBNP was statistically significant only for the 30-day death/HF rehospitalization endpoint in both unad-justed (*p* = 0.03) and adjusted (*p* = 0.02) models (Online Table 4). Specifically, there was a positive association between baseline sST2 and outcomes for high (above median) baseline NT-proBNP, and a negative association between sST2 and outcomes for

TABLE 1 Baseline Characteristics

	Baseline sST2					p Value
	Total (n = 858)	Quartile 1 (n = 215)	Quartile 2 (n = 214)	Quartile 3 (n = 214)	Quartile 4 (n = 215)	
Range, ng/ml		<48.2	48.2-71.2	71.2-111.1	≥111.1	
Age, yrs	65.5 ± 15.2	62.27 ± 15.48	64.6 ± 14.4	68.3 ± 14.82	66.9 ± 15.5	<0.001
Female, %	31.6	37.7	34.1	26.6	27.9	<0.001
White, %	67.8	60.5	67.8	69.6	73.5	<0.001
Systolic BP, mm Hg	127.3 ± 19.9	129.3 ± 21.4	129.9 ± 21.1	126.2 ± 18.8	123.8 ± 17.4	0.004
Heart rate, beats/min	80.2 ± 16.3	80.2 ± 14.3	79.5 ± 17.4	80.0 ± 17.0	81.1 ± 16.3	0.896
Atrial fibrillation, %	41.3	32.1	36.9	52.3	43.7	<0.001
Hypertension, %	78.3	83.3	81.3	75.2	73.5	<0.001
BUN, mg/dl	28.3 ± 16.8	24.0 ± 13.0	26.5 ± 15.9	30.7 ± 19.1	32.0 ± 17.5	<0.001
Creatinine, mg/dl	1.44 ± 0.6	1.34 ± 0.5	1.37 ± 0.5	1.49 ± 0.61	1.54 ± 0.6	<0.001
Sodium, mmol/l	138.6 ± 4.0	138.9 ± 3.5	139.3 ± 3.6	138.7 ± 3.8	137.6 ± 4.6	0.004
NT-proBNP (n = 752), pg/ml	5,545 (2,856-11,097)	2,917 (1,368-6,370)	4,616 (2,894-9,372)	6,134 (3,538-11,357)	9,388 (5,291-14,839)	<0.001
LVEF, %	26 (20-40)	30 (20-40)	26 (20-40)	25 (20-40)	25 (20-40)	0.280
Time from presentation to randomization, h	16.3 ± 9.4	17.26 ± 10.78	16.29 ± 8.74	14.45 ± 8.32	17.15 ± 9.27	0.387
Ischemic etiology, %	60.3	55.3	60.3	62.1	63.3	<0.001
Beta-blockers, %	75.3	73.0	74.8	77.6	75.8	<0.001
ACEi or ARB, %	64.7	65.1	67.3	62.1	64.2	<0.001
MRA, %	23.8	23.3	24.8	22.4	24.7	<0.001

Values are mean ± SD or median (interquartile range), unless otherwise indicated. All p values were from test of trend (Jonckheere-Terpstra test for continuous and Cochran-Armitage test for categorical variables).

ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; BP = blood pressure; BUN = blood urea nitrogen; LVEF = left ventricular ejection fraction; MRA = mineralocorticoid receptor antagonist; NT-proBNP = aminoterminal pro-B-type natriuretic peptide; sST2 = soluble growth stimulation expressed gene 2.

low baseline NT-proBNP (Online Figure 1). In contrast, there was no interaction between baseline sST2 and NT-proBNP for the 180-day death endpoint (p = 0.77) (Online Table 3). In particular, those with

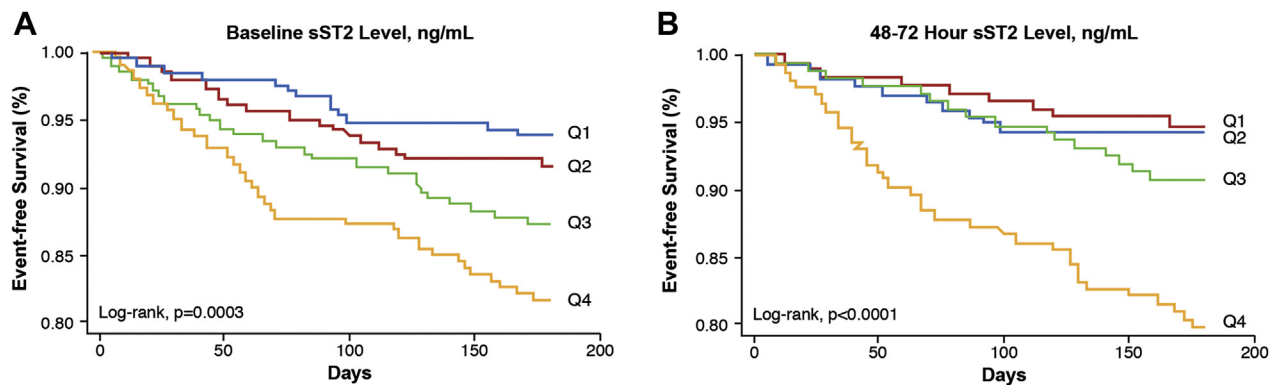
both elevated baseline sST2 and baseline NT-proBNP (stratified by their median values) portended the highest 180-day mortality risk (Online Figure 2). Cubic spline analyses also supported the linearity of

TABLE 2 Baseline sST2 Levels and Adverse Clinical Outcomes and Interactions With the ASCEND-HF Trial Risk Model and NT-proBNP

Model	30-Day Death	p Value	30-Day Death/HF Rehospitalization	p Value	180-Day Death	p Value
Baseline sST2						
Univariate model	2.30 (1.15-4.74)	0.019	1.67 (1.17-2.39)	0.005	2.21 (1.57-3.13)	<0.001
Adjusted model 1	1.95 (0.91-4.16)	0.085	1.37 (0.93-2.02)	0.117	1.91 (1.33-2.72)	<0.001
Adjusted model 2	1.52 (0.66-3.50)	0.324	1.07 (0.68-1.67)	0.775	1.35 (0.90-2.03)	0.145
Event rates	24/856 (2.8)		82/667 (12.3)		97/858 (11.3)	
48-72-h follow-up sST2						
Univariate model	1.85 (0.81-4.20)	0.145	2.11 (1.42-3.13)	<0.001	2.64 (1.82-3.84)	<0.001
Adjusted model 1	1.47 (0.61-3.59)	0.387	1.52 (0.98-2.37)	0.063	2.12 (1.42-3.16)	<0.001
Adjusted model 2	1.07 (0.40-2.86)	0.889	1.32 (0.82-2.12)	0.255	1.77 (1.14-2.74)	0.011
Event rates	16/662 (2.4)		82/667 (12.3)		97/858 (11.3)	
30-day follow-up sST2†						
Univariate model	—	—	—	—	2.29 (1.46-3.62)	<0.001
Adjusted model 1	—	—	—	—	2.29 (1.35-3.88)	0.002
Adjusted model 2	—	—	—	—	2.16 (1.22-3.80)	0.008
Event rates	—	—	—	—	41/589 (7.0)	

Values are odds ratio (95% confidence interval) or n/N (%). Both sST2 and NT-proBNP were both log transformed, increments per log increase; adjusted model 1 = ASCEND-HF (Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure) trial risk model according to endpoints (Online Table 1); adjusted model 2 = Model 1 plus NT-proBNP (with corresponding time point). †All deaths before 30 days were excluded from the 30-day follow-up analysis.

HF = heart failure; other abbreviations as in Table 1.

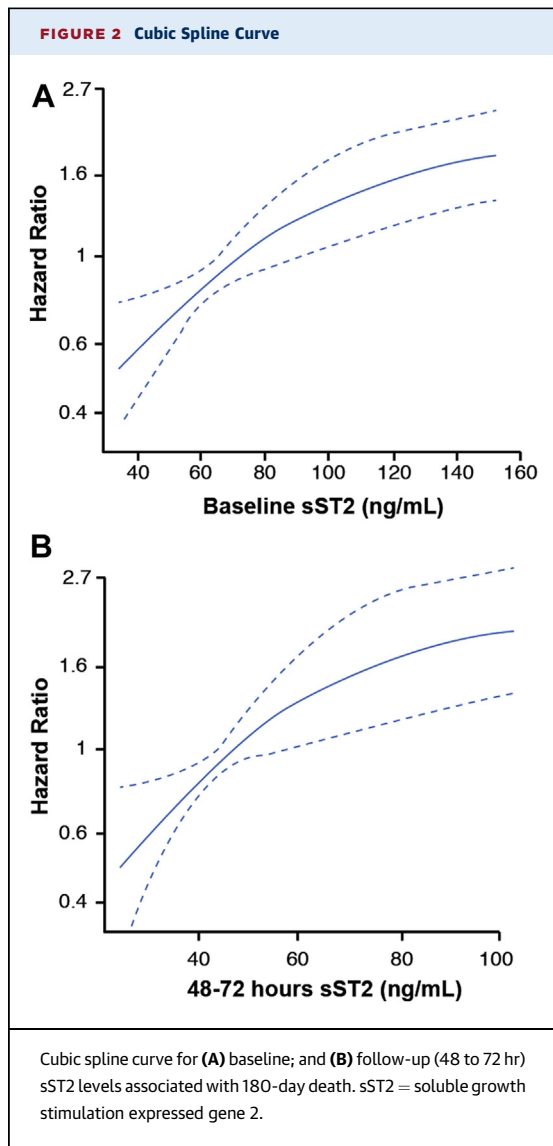
FIGURE 1 Kaplan-Meier Analysis for 180-Day Survival

Kaplan-Meier analysis for 180-day survival, stratified by quartiles of (A) baseline and (B) 48- to 72-h follow-up plasma soluble growth stimulation expressed gene 2 (sST2) levels. Baseline sST2 tertile ranges: Quartile 1 (Q1) = <48.2 ng/ml; Quartile 2 = 48.2 to 71.2 ng/ml; Quartile 3 = 71.3 to 111.2 ng/ml; Quartile 4 = >111.2 ng/ml. 48- to 72-h sST2 tertile ranges: Quartile 1 = <32.4 ng/ml; Quartile 2 = 32.4 to 46.8 ng/ml; Quartile 3 = 46.9 to 70.3 ng/ml; Quartile 4 = >70.3 ng/ml.

the 180-day mortality risk for baseline sST2 levels (Figure 2A).

FOLLOW-UP sST2 LEVELS AND PROGNOSIS. At 48 to 72 h after enrollment, higher sST2 levels portend increased risk of all-cause death at both 30 and 180 days, as well as death/rehospitalization at 30 days (Table 2). Elevated follow-up sST2 was also associated with increased risk of death/worsening HF before discharge (OR: 2.41; 95% CI: 1.25 to 4.63; $p = 0.008$). After adjustments for the ASCEND-HF risk model, the prognostic significance of follow-up sST2 levels was only relevant for 180-day death, and remained borderline significant with the addition of baseline NT-proBNP to the ASCEND-HF risk model (adjusted HR: 1.61; 95% CI: 1.00 to 2.60; $p = 0.051$) (Table 2). Examining the Kaplan-Meier curves revealed that the divergence of 180-day mortality risk occurred between the third and fourth quartile of the 48- to 72-h follow-up sST2 level (71.2 ng/ml). Furthermore, adding 48- to 72-h follow-up sST2 to the ASCEND-HF risk model, plus follow-up NT-proBNP, correctly reclassified 15.6% of subjects for the 180-day death endpoint (with 13.85% events correctly classified and 1.75% nonevents correctly classified; Online Table 3B). Cubic spline analyses supported the linearity of the risk at follow-up (Figure 2B). In addition, 30-day follow-up sST2 levels also provide incremental prognostic value in either of the adjusted models (Table 2, Online Figure 3), with similar modest reclassification to the 48- to 72-h follow-up data (Online Table 3C).

CHANGES IN sST2 LEVELS AND PROGNOSIS. Among the 858 subjects in the biomarker substudy, 680 had samples for both baseline and 48 to 72 h time points. Compared to baseline, an overall 64.4% and 51.6% reduction in absolute levels of sST2 levels occurred at 48 to 72 h and at 30 days after randomization, respectively. The median absolute change in sST2 from baseline to 48 to 72 h was -22.80 (IQR: -44.70 to -6.44) ng/ml. At 48 to 72 h, there was no lowering of sST2 absolute levels from baseline in 14.4% of subjects; this was associated with poorer outcomes, including 30-day death/HF readmission (OR: 2.50; 95% CI: 1.45 to 4.32; $p = 0.001$) and 180-day death (HR: 1.98; 95% CI: 1.15 to 3.42; $p = 0.013$) when compared with subjects showing any decrease in sST2 (Figure 3). After adjustments for the ASCEND-HF risk model and baseline NT-proBNP, the prognostic value of the lack of sST2 lowering at 48 to 72 h from baseline was significant for the outcome of 30-day death/HF readmission (adjusted OR: 1.94; 95% CI: 1.01 to 3.72; $p = 0.046$), but not for the 180-day death endpoint (adjusted HR: 1.27; 95% CI: 0.69 to 2.35; $p = 0.442$). Because the combined biologic/analytic variability for sST2 has been previously reported as ~30% (21,22), we further defined a clinically relevant sST2 reduction as a >30% decrease in sST2 levels from baseline to 48 to 72 h (which occurred in 377 subjects, or 55%). Compared to those with a $\leq 30\%$ sST2 reduction, subjects who demonstrated a >30% reduction in sST2 had lower event rates in all endpoints except for 30-day death (Online Figure 4).

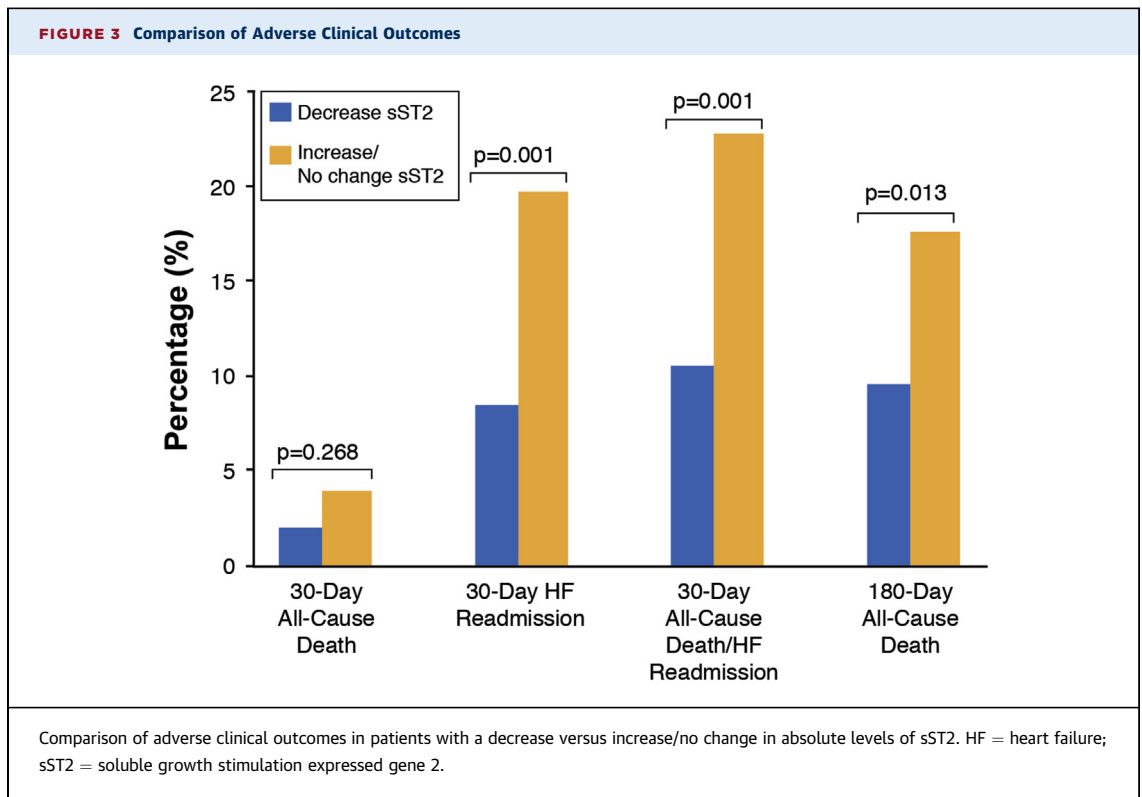


To further examine whether there is a threshold of follow-up sST2 level that conferred heightened risk, [Online Table 5](#) outlines the baseline characteristics of subgroups according to changes from baseline to 48- to 72-h sST2 levels, stratified at a baseline median sST2 level of 71.2 ng/ml ([Online Figure 5](#) presents the CONSORT diagram for subgroup distributions). In the cohort with elevated baseline sST2 levels (>71.2 ng/ml), we further observed a 3-fold increase in 180-day mortality risk between those with persistently high sST2 (>71.2 ng/ml) versus low (\leq 71.2 ng/ml) at 48- to 72-h follow-up ([Figure 4](#), also [Online Figure 6](#) for all subgroups); this finding remained statistically significant in multivariate analysis after adjusting for the ASCEND-HF risk model and baseline NT-proBNP ([Table 3](#)).

CHANGES IN sST2 LEVELS AND TREATMENT. Overall, 502 subjects (257 assigned to nesiritide, 245 assigned to placebo) had samples collected at all 3 time points. There were no significant differences in baseline characteristics between the nesiritide and placebo treatment groups, including similar mean NT-proBNP levels ($8,910 \pm 10,492$ pg/ml vs. $8,968 \pm 9,577$ pg/ml; $p = 0.329$). Both groups demonstrated a significant reduction in sST2 levels from baseline to 48- to 72-h follow-up, and further lowering of sST2 levels was observed at the 30-day visit in both groups ([Table 4](#)). The absolute changes in sST2 from baseline to 48 to 72 h was significantly greater in the placebo group than in the nesiritide group (respective median absolute changes -26.11 ng/ml vs. -18.05 mg/l; $p = 0.005$), but the 2 groups did not differ by treatment regarding absolute changes in sST2 from baseline to 30 days ([Table 4](#)). Additionally, sST2 levels at 30-day follow-up and absolute changes in sST2 levels from baseline to 30 days were similar between the 2 treatment groups ([Table 4](#)).

DISCUSSION

There are 4 major findings from this study. First, we observed that baseline sST2 levels elevated in the acute HF setting were comparable with earlier reports ([23-26](#)), and were higher than those reported in the chronic setting (cutoff at 35 ng/ml) ([9,15](#)). Second, the prognostic findings for sST2 at baseline for 180-day outcomes were generally neutral after adjustments for the ASCEND-HF risk model and NT-proBNP, despite the significant univariate findings. In contrast, follow-up (48 to 72 h or 30 days) sST2 appeared to provide incremental prognostic value, albeit diminished following covariate and NT-proBNP adjustments. Third, consistent with previous reports sST2 levels tend to fall after medical therapy ([23,27,28](#)), but we found that 1 in 7 patients demonstrated no fall in sST2 levels following medical therapy. Meanwhile, persistently elevated sST2 levels (above baseline median of 71.2 ng/ml), or lack of any lowering of sST2 levels despite medical therapy, may define a higher-risk subset of patients compared to those who demonstrated a fall in sST2 level following medical therapy as seen in a smaller series ([23](#)). Finally, contrary to our hypothesis, nesiritide did not demonstrate any significant effects on lowering sST2 levels over standard therapy in the long-term. Conversely, the placebo group showed a greater fall in sST2 levels from baseline to 48 to 72 h than the nesiritide group, even though such difference did not extend to the 30-day timepoint. Therefore, persistently



elevated sST2 following stabilization during acute HF hospitalization may identify a higher risk cohort even after clinical risk factors and NT-proBNP levels have been considered.

The lack of incremental prognostic significance of baseline sST2 with the addition of NT-proBNP levels to the standard ASCEND-HF risk model was

unexpected, because previous studies have demonstrated an incremental prognostic value of sST2 levels—even when adjusting for the levels of various natriuretic peptide assays (6,16,29). Although there are some inconsistencies between the Cox models and the reclassification analysis, it has been increasingly recognized that the latter may in some cases

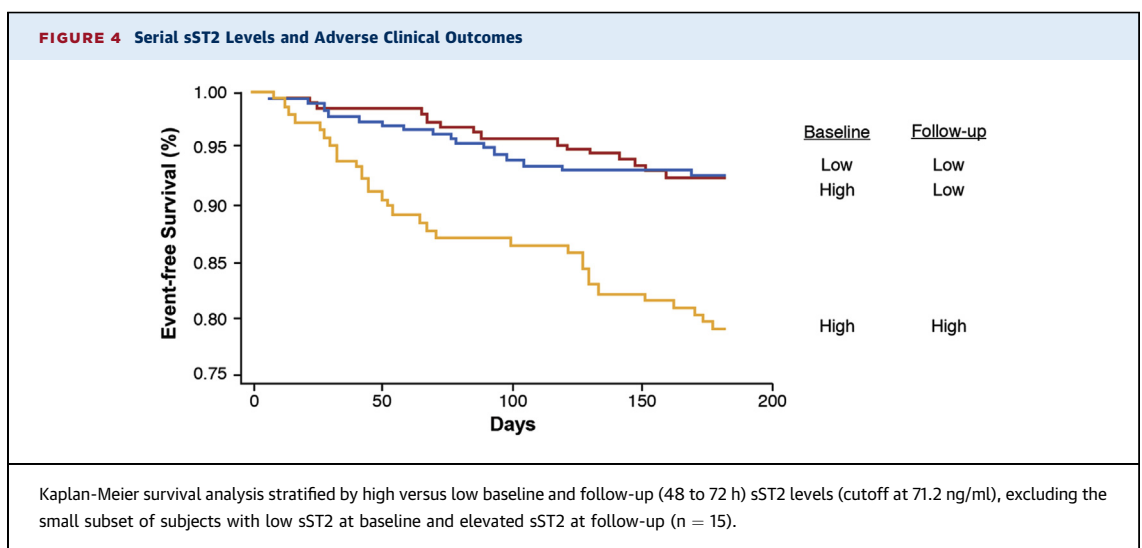


TABLE 3 HRs for Death at 180 Days by Change Trends in sST2 From Baseline to 48-72 h (Using Median sST2 of 71.2 ng/ml as Cutoff)

	High Versus Low sST2 at Baseline; Low at 48-72-h Follow-Up			High at Baseline; High Versus Low sST2 at 48-72-h Follow-Up		
	Low→Low	High→Low	p Value	High→Low	High→High	p Value
Unadjusted HR	1.0	1.03 (0.54-1.98)	0.924	1.0	3.01 (1.63-5.59)	<0.001
Adjusted HR (model 1)	1.0	0.82 (0.42-1.59)	0.552	1.0	2.60 (1.38-4.89)	0.003
Adjusted HR (model 2)	1.0	0.66 (0.34-1.29)	0.226	1.0	2.42 (1.27-4.61)	0.007
Event rates	23/315 (7.3)	15/196 (7.7)		15/196 (7.7)	31/147 (21.1)	

Values are HR (95% confidence interval) or n/n (%).
 HR = hazard ratio; other abbreviations can be found in Table 1.

overestimate the incremental value of a biomarker even in independent validation data (30). Interestingly, many of the earlier studies that conducted multivariate analyses had limited covariate(s) or single cutoff values, and the majority of these studies conducted utilized research-based assays (6,8,29). Also, most previous studies had a more extended period of follow-up beyond 180 days (6,8,16), and did not include blood urea nitrogen, which is a widely available and robust prognostic covariate (31). Furthermore, in a clinical trial population such as the ASCEND-HF trial, there were specific inclusion and exclusion criteria, where a number of extreme phenotypes would have been excluded. The lower comorbidity in a clinical trial population than in single-center observational cohorts and the cardiac nonspecific nature of sST2 (7,21,32) might have also tracked better with long-term adverse outcomes than intermediate adverse outcomes following hospital discharge from acute HF. Nevertheless, our findings corroborate 2 recent post-hoc biomarker analysis from well-characterized large clinical trials of chronic HF, both of which observed that the prognostic value of sST2 was less robust when natriuretic peptide levels were included in the multivariate models (9,33). In fact, recent studies that measure transcardiac gradient of sST2 levels have even challenged the cardiac origin of circulating sST2 (7,34). Because natriuretic peptide testing is so widely available and its clinical utility for diagnosis and prognosis in the setting of acute HF has been well established, further studies that explore the incremental value of sST2 testing in a multimarker strategy with natriuretic peptides are warranted before broad clinical adoption.

Because insights can be gained not only from the absolute circulating ST2 levels, but from changes following medical stabilization, we compared subjects that did not show a reduction in sST2 levels (1 of 7 subjects in our cohort) versus subjects who did. As reported in the published data, one of the

advantages of sST2 is the relatively low assay and biological variability compared with other cardiac biomarkers, which may favor its reliability in serial testing (22,35). Previous studies have demonstrated that either a 15% reduction in sST2 or a lower sST2 ratio (<75%) within 2 weeks was observed in destabilized HF patients with no subsequent events compared to those with events (27). Our sensitivity analyses (using both a clinically relevant sST2 reduction of >30% or below a threshold of 60 ng/ml) further demonstrate the prognostic importance of lowering sST2 levels in those with elevated baseline sST2, and a 4-fold increase in mortality risk between those with sST2 levels above versus below 60 ng/ml at 48- to 72-h follow-up (Online Figure 4). The observed ranges were similar to sST2 levels measured in a smaller cohort with serial samples measured at baseline and at day 4 (23).

The lack of long-term differences in absolute changes of sST2 levels over time between nesiritide and placebo is consistent with the primary results of the ASCEND-HF trial. In fact, the short-term reduction in absolute levels of sST2 appeared to be significantly larger in the placebo group, even though both groups achieved similar urine volumes and similar median blood pressures or rates of hypotension.

TABLE 4 Impact of Nesiritide Therapy on Absolute Changes in sST2 Levels

sST2 Levels (ng/ml)	Placebo (n = 245)	Nesiritide (n = 257)	p Value
Baseline	70.69 (51.40 to 102.54)	69.24 (46.86 to 108.05)	0.567
48-72 h	42.13 (30.85 to 60.81)	48.46 (32.52 to 67.67)	0.067
30 days	39.25 (28.12 to 61.94)	39.74 (27.52 to 67.20)	0.590
Changes from baseline to 48-72 h	-26.11 (-45.88 to -12.03)	-18.05 (-41.20 to -4.37)	0.005
Changes from baseline to 30 days	-26.26 (-52.10 to -6.13)	-21.01 (-52.27 to -2.62)	0.26

p value from nonparametric test.
 Abbreviations as in Table 1.

STUDY STRENGTHS. The strengths of this study include: 1) meticulously collected serial samples in a prospective biomarker study in a large representative patient population; 2) adjudicated endpoints including HF rehospitalizations and dyspnea relief as part of a multicenter randomized clinical trial; and 3) a large study population compared to previous studies using the Food and Drug Administration-cleared assay.

STUDY LIMITATIONS. The number of events, relatively small size of the study groups (particularly with subgroup analyses), and relatively short (180-day) mortality endpoint may have reduced the power to detect the incremental prognostic value of sST2. Given our present findings from this post-hoc analysis, the incremental value of sST2 testing in a multimarker strategy with natriuretic peptides may depend on the appropriate timing (at follow-up rather than at baseline) and patient population (in those with high rather than low NT-proBNP levels); this should be further investigated. Furthermore, the clinical relevance of assessing changes in sST2 should be further investigated in these patient subsets.

CONCLUSIONS

Elevated levels of sST2 at baseline and follow-up were associated with an increased risk of adverse clinical events. However, the addition of baseline

sST2 to a standard risk model plus NT-proBNP levels did not improve the prediction of 180-day outcomes, yet failure to lower sST2 levels portends a poor prognosis. Nesiritide did not demonstrate any significant effects on lowering sST2 levels over standard therapy.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE:

Elevated sST2 levels at follow-up, but not at baseline, provided incremental prognostic value for 180-day mortality beyond clinical covariates and natriuretic peptide levels.

TRANSLATIONAL OUTLOOK:

Further studies that explore the incremental value and timing of sST2 testing in a multimarker strategy with natriuretic peptides are warranted before broad clinical adoption. In the interim, the lack of incremental reduction in sST2 by nesiritide also calls into question the role of myocardial stress as the underlying mechanisms of sST2 generation in the setting of heart failure.

REFERENCES

- Januzzi JL Jr. ST2 as a cardiovascular risk biomarker: from the bench to the bedside. *J Cardiovasc Transl Res* 2013;6:493-500.
- Karayannis G, Triposkiadis F, Skoularigis J, Georgoulas P, Butler J, Giamouzis G. The emerging role of Galectin-3 and ST2 in heart failure: practical considerations and pitfalls using novel biomarkers. *Curr Heart Fail Rep* 2013;10:441-9.
- Weinberg EO, Shimpo M, Hurwitz S, Tominaga S, Rouleau JL, Lee RT. Identification of serum soluble ST2 receptor as a novel heart failure biomarker. *Circulation* 2003;107:721-6.
- Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J Clin Invest* 2007;117:1538-49.
- Shimpo M, Morrow DA, Weinberg EO, et al. Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction. *Circulation* 2004;109:2186-90.
- Januzzi JL Jr., Peacock WF, Maisel AS, et al. Measurement of the interleukin family member ST2 in patients with acute dyspnea: results from the PRIDE (Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study. *J Am Coll Cardiol* 2007;50:607-13.
- Bartunek J, Delrue L, Van Durme F, et al. Non-myocardial production of ST2 protein in human hypertrophy and failure is related to diastolic load. *J Am Coll Cardiol* 2008;52:2166-74.
- Rehman SU, Mueller T, Januzzi JL Jr. Characteristics of the novel interleukin family biomarker ST2 in patients with acute heart failure. *J Am Coll Cardiol* 2008;52:1458-65.
- Felker GM, Fiuzat M, Thompson V, et al. Soluble ST2 in ambulatory patients with heart failure: Association with functional capacity and long-term outcomes. *Circ Heart Fail* 2013;6:1172-9.
- Shah RV, Chen-Tournoux AA, Picard MH, van Kimmenade RR, Januzzi JL. Serum levels of the interleukin-1 receptor family member ST2, cardiac structure and function, and long-term mortality in patients with acute dyspnea. *Circ Heart Fail* 2009;2:311-9.
- Daniels LB, Clopton P, Iqbal N, Tran K, Maisel AS. Association of ST2 levels with cardiac structure and function and mortality in outpatients. *Am Heart J* 2010;160:721-8.
- Weir RA, Miller AM, Murphy GE, et al. Serum soluble ST2: a potential novel mediator in left ventricular and infarct remodeling after acute myocardial infarction. *J Am Coll Cardiol* 2010;55:243-50.
- Ahmad T, Fiuzat M, Neely B, et al. Biomarkers of myocardial stress and fibrosis as predictors of mode of death in patients with chronic heart failure. *J Am Coll Cardiol HF* 2014;2:260-8.
- Pascual-Figal DA, Ordonez-Llanos J, Tormel PL, et al. Soluble ST2 for predicting sudden cardiac death in patients with chronic heart failure and left ventricular systolic dysfunction. *J Am Coll Cardiol* 2009;54:2174-9.
- Ky B, French B, McCloskey K, et al. High-sensitivity ST2 for prediction of adverse outcomes in chronic heart failure. *Circ Heart Fail* 2011;4:180-7.
- Lassus J, Gayat E, Mueller C, et al. Incremental value of biomarkers to clinical variables for mortality prediction in acutely decompensated heart failure: the Multinational Observational Cohort on Acute Heart Failure (MOCA) study. *Int J Cardiol* 2013;168:2186-94.
- O'Connor CM, Starling RC, Hernandez AF, et al. Effect of nesiritide in patients with acute

decompensated heart failure. *N Engl J Med* 2011; 365:32-43.

18. Harrell FE Jr. *Regression Modeling Strategies*. New York, NY: Springer, 2001.

19. Pencina MJ, D'Agostino RB Sr., D'Agostino RB Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med* 2008;27: 157-72, discussion 207-12.

20. Pencina MJ, D'Agostino RB Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med* 2011;30:11-21.

21. Dieplinger B, Januzzi JL Jr., Steinmair M, et al. Analytical and clinical evaluation of a novel high-sensitivity assay for measurement of soluble ST2 in human plasma—the Presage ST2 assay. *Clin Chim Acta* 2009;409:33-40.

22. Wu AH, Wians F, Jaffe A. Biological variation of galectin-3 and soluble ST2 for chronic heart failure: implication on interpretation of test results. *Am Heart J* 2013;165:995-9.

23. Manzano-Fernandez S, Januzzi JL, Pastor-Perez FJ, et al. Serial monitoring of soluble interleukin family member ST2 in patients with acutely decompensated heart failure. *Cardiology* 2012; 122:158-66.

24. Gruson D, Lepoutre T, Ahn SA, Rousseau MF. Increased soluble ST2 is a stronger predictor of long-term cardiovascular death than

natriuretic peptides in heart failure patients with reduced ejection fraction. *Int J Cardiol* 2014;172: e250-2.

25. Gaggin HK, Szymonifka J, Bhardwaj A, et al. Head-to-head comparison of serial soluble ST2, growth differentiation factor-15, and highly-sensitive troponin T measurements in patients with chronic heart failure. *J Am Coll Cardiol HF* 2014;2:65-72.

26. Bayes-Genis A, de Antonio M, Vila J, et al. Head-to-head comparison of 2 myocardial fibrosis biomarkers for long-term heart failure risk stratification: ST2 versus galectin-3. *J Am Coll Cardiol* 2014;63:158-66.

27. Boisot S, Beede J, Isakson S, et al. Serial sampling of ST2 predicts 90-day mortality following destabilized heart failure. *J Card Fail* 2008;14:732-8.

28. Breidhardt T, Balmelli C, Twerenbold R, et al. Heart failure therapy-induced early ST2 changes may offer long-term therapy guidance. *J Card Fail* 2013;19:821-8.

29. Pascual-Figal DA, Manzano-Fernandez S, Boronat M, et al. Soluble ST2, high-sensitivity troponin T- and N-terminal pro-B-type natriuretic peptide: complementary role for risk stratification in acutely decompensated heart failure. *Eur J Heart Fail* 2011;13:718-25.

30. Kerr KF, Wang Z, Janes H, McClelland RL, Psaty BM, Pepe MS. Net reclassification indices for evaluating risk-prediction instruments: a critical review. *Epidemiology* 2014;25:114-21.

31. Tang WH, Dupont M, Hernandez AF, et al. Comparative assessment of short-term adverse events in acute heart failure with cystatin C and other estimates of renal function: results from the ASCEND-HF trial. *J Am Coll Cardiol HF* 2015;3: 40-9.

32. Pascual-Figal DA, Januzzi JL. The biology of ST2: the International ST2 Consensus Panel. *Am J Cardiol* 2015;115:3B-7B.

33. Anand IS, Rector TS, Kuskowski M, Snider J, Cohn JN. Prognostic value of soluble ST2 in the Valsartan Heart Failure Trial. *Circ Heart Fail* 2014; 7:418-26.

34. Kaye DM, Mariani JA, van Empel V, Maeder MT. Determinants and implications of elevated soluble ST2 levels in heart failure. *Int J Cardiol* 2014;176:1242-3.

35. Wojtczak-Soska K, Sakowicz A, Pietrucha T, Lelonek M. Soluble ST2 protein in the short-term prognosis after hospitalization in chronic systolic heart failure. *Kardiol Pol* 2014;72: 725-34.

KEY WORDS acute decompensated heart failure, nesiritide, prognosis, soluble ST2

APPENDIX For supplemental tables and figures, please see the online version of this article.