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

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Objectives

The aim of this study was to examine the value of measurement of the interleukin-1 receptor family member ST2 in patients with dyspnea.

Background

Concentrations of ST2 have been reported to be elevated in patients with heart failure (HF).

Methods

Five hundred ninety-three dyspneic patients with and without acute destabilized HF presenting to an urban emergency department were evaluated with measurements of ST2 concentrations. Independent predictors of death at 1 year were identified.

Results

Concentrations of ST2 were higher among those with acute HF compared with those without (0.50 vs. 0.15 ng/ml; p < 0.001), although amino-terminal pro-brain natriuretic peptide (NT-proBNP) was superior to ST2 for diagnosis of acute HF. Median concentrations of ST2 at presentation to the emergency department were higher among decedents than survivors at 1 year (1.08 vs. 0.18 ng/ml; p < 0.001), and in multivariable analyses, an ST2 concentration ≥0.20 ng/ml strongly predicted death at 1 year in dyspneic patients as a whole (HR = 5.6, 95% confidence interval [CI] 2.2 to 14.2; p < 0.001) as well as those with acute HF (hazard ratio [HR] = 9.3, 95% CI 1.3 to 17.8; p = 0.03). This risk associated with an elevated ST2 in dyspneic patients with and without HF appeared early and was sustained at 1 year after presentation (log-rank p value <0.001). A multi-marker approach with both ST2 and NT-proBNP levels identified subjects with the highest risk for death.

Conclusions

Among dyspneic patients with and without acute HF, ST2 concentrations are strongly predictive of mortality at 1 year and might be useful for prognostication when used alone or together with NT-proBNP.

The ST2 gene, a member of the interleukin–1 receptor family (1–3), was recently described to be markedly up-regulated in an experimental model of heart failure (HF) (4). The ST2 is thought to bind interleukin-33, another hormone induced and released after stretch of cardiomyocytes (5,6), and interruption of the ST2 gene results in progressive myocardial fibrosis and hypertrophy in experimental models (5). Circulating ST2 concentrations might be prognostically meaningful in those with chronic severe HF (7) or acute myocardial infarction (MI) (8), but data regarding measurement of ST2 in cardiac disease states were generally limited in scope. Thus, we wished to examine the
association(s) between concentrations of ST2 and the presence of acute HF in dyspneic patients, as well as the relationship between ST2 concentrations and outcomes in these subjects. To do so, we analyzed blood samples from the recently reported PRIDE (Pro–Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department) study (9).

Methods

The PRIDE study. The PRIDE study was a prospective, blinded study of 599 dyspneic subjects presenting to the emergency department of the Massachusetts General Hospital and was performed for the purpose of validation of the use of amino-terminal pro-brain natriuretic peptide (NT-proBNP) testing (Elecsys ProBNP, Roche Diagnostics, Indianapolis, Indiana). Other analytes tested in the PRIDE study included C-reactive protein (CRP), with a high-sensitivity assay (Roche Diagnostics).

As reported (9), 209 subjects (35%) in the PRIDE study were adjudicated to have dyspnea due to acute destabilized HF, and ascertainment of 1 year vital status was complete in 99.3% (10).

ST2 analysis. Blood collected at the time of presentation was analyzed for concentrations of ST2 with an enzyme-linked immunosorbent assay (Medical & Biological Laboratories Co., Woburn, Massachusetts) with characteristics previously described (11). This assay uses monoclonal antibodies to human ST2 for both capture and detection and had an inter-assay coefficient of variation of 17.5% in the present analysis. The blood used for the present study had been previously subjected to a single freeze-thaw cycle.

Statistical analyses. UNIVARIABLE AND MULTIVARIABLE LINEAR REGRESSION ANALYSES. For correlation studies, ST2, CRP, and NT-proBNP results were log-transformed to establish normal distribution. Correlations between log-transformed ST2, CRP, and NT-proBNP concentrations were evaluated with the Spearman correlation coefficient; multivariable linear regression analyses were also performed, with log-transformed ST2 concentrations as the dependent variable.

COMPARISONS BETWEEN GROUPS. Comparisons of clinical characteristics between patients were performed with chi-square tests for categorical data; and for non-normally distributed continuous variables, the Wilcoxon rank-sum test was used.

CUT-POINT ANALYSES FOR HF DIAGNOSIS. Receiver-operating characteristic (ROC) curve analysis with Analyse-It software (Analyse-It Ltd., Leeds, United Kingdom) was performed for ST2, with diagnosis of acute HF as the reference standard, and area under the curve (AUC) for this purpose was estimated; as well, candidate ST2 cut-points for HF diagnosis were identified.

ST2 and prognosis. Patients were divided into ST2 deciles, and the frequency of mortality relative to increasing ST2 concentrations above or below the median were estimated with odds ratios (OR) with 95% confidence intervals (CI).

To evaluate ST2 for prediction of death in dyspneic patients, ROC analyses with death at 1 year as the reference standard were performed, and candidate cut-points for mortality prediction were identified. After, candidate ST2 cut-points for prognosis (on the basis of cut-points from diagnostic and prognostic ROC as well as decile analyses) were evaluated against other candidate variables for prognosis with bootstrapping techniques. Multiple bootstrap runs of the STATA (STAT Corp., College Station, Texas) stcox program were run to narrow down the candidate set of significant independent variables that were explanatory for death. The bootstrap sample size was 593 (the size of the entire data set).

Factors entered into the bootstrap analysis included variables from history, physical examination, and laboratory testing. Independent variables that seemed significantly related to death in <70% of 1,000 subsamples were eliminated as spurious. After bootstrap iterations, the top-selected (i.e., those selected in ≥80 bootstrap iterations) variables were entered into a Cox proportional hazards model; hazards ratios (HR) were generated, and the STATA bootstrapstcox prefix was used in the final run of the best predictors of death (selected in the aforementioned manner) to estimate 95% CI for the coefficients of these predictors in the final fitted Cox proportional hazards model.

In a secondary analysis, the relative importance of candidate prognostic factors was examined in those patients with acute HF; this model included age, ST2, CRP, and NT-proBNP values, measures of renal function (12), symptom severity, and ejection fraction.

Hazard curves compared mortality rates across the year after presentation in groups divided as a function of ST2 concentrations with the log-rank test to identify significance. Lastly, to examine the additive value of ST2 relative to NT-proBNP, we examined rates of death at 1 year as a function of values for ST2 + NT-proBNP in all subjects as well as those with acute HF.

For all statistical analyses, either SPSS (SPSS Inc., Chicago, Illinois) or STATA software (Stata Corp., College Station, Texas) was used; all p values are 2-sided, with composite results <0.05 considered significant.
Results

Of 599 subjects in the original PRIDE cohort, 593 had blood samples available for analysis; of these, 208 had acute HF. At 1 year, 93 subjects had died; the characteristics of decedents versus survivors at 1 year are depicted in Table 1.

Correlations. In all subjects, there was a modest correlation between concentrations of log-transformed ST2 and CRP (r = 0.54, p < 0.001) or NT-proBNP (r = 0.58, p < 0.001). In multivariable linear regression analyses, several variables were found to be significantly directly or inversely related to ST2 concentrations (all p < 0.05), including historical factors (prior obstructive airways disease, T = 2.29), HF symptom severity (as judged by the New York Heart Association [NYHA] functional classification; T = 4.04), vital signs (fever, T = 4.51; pulse rate, T = 8.21), or atrial fibrillation at presentation (T = 8.21) as well as results of diagnostic testing, including serum creatinine (T = 3.55), CRP (T = 7.85) and the presence of infiltrates on chest radiography (T = 3.96). The NT-proBNP was the most strongly associated variable with ST2 concentrations (T = 10.96; p < 0.001).

Biomarker concentrations as a function of HF diagnosis.

The median ST2 concentration in the group as a whole (n = 593) was 0.23 ng/ml (interquartile range [IQR] 0.09 to 0.68 ng/ml). Concentrations of ST2 were significantly higher in those dyspneic patients judged to have acute HF (0.50 ng/ml, IQR 0.27 to 1.22 ng/ml) versus those without (0.15 ng/ml, IQR 0.06 to 0.42 ng/ml; p < 0.001). Among those subjects with acute HF and available data regarding left ventricular systolic function (n = 202, 97% of acute HF subjects), those with impaired left ventricular systolic function (n = 85) had higher median concentrations of ST2 (0.67 ng/ml; IQR 0.31 to 1.50 ng/ml) compared with those subjects with non-systolic HF (n = 117; 0.42 ng/ml, IQR 0.22 to 0.90 ng/ml; p = 0.012). On the basis of ROC analyses, the optimal ST2 cut-point was 0.20 ng/ml; ST2 had an AUC of 0.74 for the diagnosis of acute HF (95% CI 0.70 to 0.78; p < 0.001), inferior to that reported for NT-proBNP from this cohort (9).

Among patients with elevated NT-proBNP dichotomized with an ST2 value 0.20 ng/ml, it was possible to detect several differences. Those with elevated NT-proBNP but low ST2 were less likely to have acute HF than those with elevated ST2 (58% vs. 81%; p = 0.001), and among those with HF, these subjects had less prevalent symptoms/signs of HF and less severe dyspnea (mean NYHA functional class 3.0 vs. 3.5; p = 0.007). As well, among those with low ST2 but elevated NT-proBNP, numerous differences in laboratory results were evident, including lower creatinine (1.2 vs. 1.4 mg/dl; p = 0.02), lower glucose levels (124 vs. 151 mg/dl; p = 0.001), and lower CRP values (19 vs. 60 mg/l; p < 0.001).

Biomarker concentrations relative to survival at 1 year in dyspneic patients. At 1 year, 93 subjects (15.7%) had died. The median concentrations of ST2 were significantly higher

### Table 1: Characteristics of Dyspneic Emergency Department Patients With Dyspnea According to Survival Status at 1 Year

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Deceased (n = 93)</th>
<th>Alive (n = 500)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD), yrs</td>
<td>72 ± 14</td>
<td>61 ± 17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender</td>
<td>51%</td>
<td>51%</td>
<td>0.97</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior heart failure</td>
<td>46%</td>
<td>21%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>62%</td>
<td>47%</td>
<td>0.008</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>12%</td>
<td>5%</td>
<td>0.01</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>38%</td>
<td>26%</td>
<td>0.02</td>
</tr>
<tr>
<td>Dyspnea at rest</td>
<td>57%</td>
<td>34%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical examination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jugular venous distension</td>
<td>15%</td>
<td>8%</td>
<td>0.03</td>
</tr>
<tr>
<td>Murmur</td>
<td>22%</td>
<td>9%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lower extremity edema</td>
<td>37%</td>
<td>22%</td>
<td>0.002</td>
</tr>
<tr>
<td>Rales</td>
<td>37%</td>
<td>24%</td>
<td>0.008</td>
</tr>
<tr>
<td>Chest radiographic findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitial edema</td>
<td>29%</td>
<td>15%</td>
<td>0.001</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>40%</td>
<td>13%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cephalization of vessels</td>
<td>3%</td>
<td>0.4%</td>
<td>0.005</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dl), mean ± SD</td>
<td>1.35 ± 0.53</td>
<td>1.06 ± 0.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/1.73 m²), mean ± SD</td>
<td>60.2 ± 36.0</td>
<td>76.5 ± 28.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl), mean ± SD</td>
<td>31.4 ± 18.7</td>
<td>19.9 ± 12.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cTnT (ng/dl), mean ± SD</td>
<td>0.12 ± 0.17</td>
<td>0.22 ± 0.65</td>
<td>0.76</td>
</tr>
<tr>
<td>NT-proBNP (pg/ml), median (interquartile range)</td>
<td>3.277 (1,086–9,868)</td>
<td>299 (71–1,807)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ST2 (ng/ml), median (interquartile range)</td>
<td>1.03 (0.88–2.43)</td>
<td>0.18 (0.08–0.51)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* cTnT = cardiac troponin T; NT-proBNP = amino-terminal pro-brain natriuretic peptide.
among decedents than survivors (1.03 [IQR 0.38 to 2.43] vs. 0.18 ng/ml [IQR 0.08 to 0.51 ng/ml]; p < 0.001) (Fig. 1). This pattern of higher ST2 concentrations in decedents remained when subjects were considered as a function of the absence (1.14 vs. 0.13 ng/ml; p < 0.001) or presence (0.90 vs. 0.45 ng/ml; p < 0.001) of acute HF.

In decile analyses, rising rates of death with ST2 concentrations >0.23 ng/ml were observed (Fig. 2), with a subsequently graded relationship between ST2 concentrations and the likelihood for death. Subjects above the ST2 median had 11-fold greater odds for death (95% CI 5.5 to 21.4; p < 0.0005) compared with those below the median.

The ROC analyses demonstrated an AUC of 0.80 (95% CI 0.75 to 0.84; p < 0.001) for ST2 and 1 year mortality (Fig. 3), with a candidate cut-point of 0.29 ng/ml.

In ROC analyses, CRP had AUC of 0.76 (p < 0.001) for 1-year mortality, which was comparable to NT-proBNP in this cohort (10).

Comparatively, in mortality analyses, ST2 had significantly more AUC compared with both NT-proBNP and CRP (p < 0.05 for both).

In a bootstrap model for prediction of death at 1 year, an ST2 concentration ≥0.20 ng/ml was most highly selected cut-point (in 96 of 100 bootstrap replications), strongly predicting death at 1 year in breathless patients in Cox proportional hazards analyses (HR = 5.6, 95% CI 2.2 to 14.2; p < 0.001). With the inclusion of NT-proBNP, an ST2 ≥0.20 ng/ml remained highly selected in bootstrap replications (86 of 100 selections), remaining strongly predictive of death at 1 year (HR = 4.6, 95% CI 1.8 to 11.8; p = 0.002) (Table 2). No first-order interactions between ST2 and NT-proBNP were identified.

In a bootstrapped logistic model for death including both ST2 and NT-proBNP, the AUC was 0.80 (95% CI 0.76 to 0.84); removing ST2 from the model resulted in an AUC of 0.72 (95% CI 0.68 to 0.77), whereas removing NT-proBNP resulted in an AUC of 0.74 (95% CI 0.71 to 0.77). The increment in either AUC from the addition of ST2 or NT-proBNP to each other was significant (both p < 0.001). Among all subjects, CRP was not selected as a
Significant predictor of death in the presence of ST2 and NT-proBNP.

In a stratified analysis of only those patients with acute HF and available ST2 results (n = 208), independent predictors of death included age (HR = 1.03; 95% CI 1.01 to 1.06, \( p = 0.009 \)), CRP \( \geq 13.5 \) mg/l (HR = 1.8; 95% CI 1.01 to 3.3, \( p = 0.05 \)), ST2 \( \geq 0.20 \) ng/ml (HR = 9.3; 95% CI 1.3 to 17.8, \( p = 0.03 \)), and NT-proBNP \( \geq 986 \) pg/ml (HR 4.95; 95% CI 1.1 to 45.4, \( p = 0.05 \)).

Among all subjects, in those with ST2 concentrations \( \geq 0.20 \) ng/ml, rates of death rose rapidly from enrollment and continued to rise at 1 year. Similar relationships between ST2 values \( \geq 0.20 \) ng/ml were seen in those without and with the diagnosis of acute HF at presentation (log-rank p value for both <0.001) (Fig. 4).

In light of the additive value of ST2 and NT-proBNP in terms of overall mortality prediction, crude rates of death in dyspneic subjects as a function of ST2 and NT-proBNP concentrations were examined. Those with low ST2 concentrations had low rates of death, irrespective of NT-proBNP concentration (Fig. 5A). The majority of mortality among subjects in the PRIDE study occurred in those with elevated ST2 concentrations; those with elevations in both ST2 and NT-proBNP had the highest rates of death.

Among those with low NT-proBNP but elevated ST2 (n = 125) there were 16 deaths (12.8%). In this cohort of subjects, the most common cause of death was from a pulmonary cause, including obstructive airway disease in 5, pneumonia in 4, lung carcinoma in 3, and pulmonary embolism in 2. Sepsis was the cause of death in 1 subject in this group, and the cause of death was unavailable in 1 subject.

Considering those patients with acute HF, similar to the group as a whole, a dramatic relationship between ST2, NT-proBNP, and outcomes was observed: in this setting, an NT-proBNP \( \geq 986 \) pg/ml was present in 184 subjects; in this group, there were 56 deaths. All but 1 of the decedents within this group had an ST2 \( \geq 0.20 \) ng/ml (Fig. 5B).

### Table 2

Identification of Independent Predictors of Death at 1 Year in Dyspneic Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bootstrap Analysis</th>
<th>Cox Proportional Hazards Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>ST2 ( \geq 0.20 ) ng/ml</td>
<td>86</td>
<td>4.6</td>
</tr>
<tr>
<td>NT-proBNP ( \geq 986 ) pg/ml</td>
<td>85</td>
<td>2.3</td>
</tr>
<tr>
<td>Cough</td>
<td>80</td>
<td>0.60</td>
</tr>
<tr>
<td>Pleural effusion on chest X-ray</td>
<td>80</td>
<td>1.6</td>
</tr>
</tbody>
</table>

The results of 100 bootstrap replications followed by Cox proportional hazards analysis are shown in the absence and presence of amino-terminal pro-brain natriuretic peptide (NT-proBNP) results in the model. Only those variables selected \( > 70/100 \) times in bootstrap replications are shown.

#### Discussion

The ST2 protein is found both as a trans-membrane and a soluble form in serum. The trans-membrane form of ST2 is thought to play a role in modulating responses of T helper type 2 cells (6) and might play a role in development of immunologic tolerance (13), whereas the soluble form of ST2 is up-regulated in growth-stimulated fibroblasts (3). Despite the potential role played by ST2 in inflammation, significant parallels between ST2 and natriuretic peptides exist: the ST2 gene is markedly up-regulated in states of myocyte stretch (4), similar to the induction of the BNP gene (14), and, in analogy to the phenotype seen in BNP-deficient mice (15), mice deficient in ST2 develop dilated and hypertrophied left ventricles, lower ejection fractions, and reduced survival (5). Why a marker with a putative role in the inflammatory system would have such parallels to natriuretic peptides is unknown, but this raises...
the possibility for a pluripotent role for ST2, representing a bridge between inflammatory and neurohormonal systems.

The ligand for ST2 was recently identified as interleukin-33, a product released by endothelia and fibroblasts in response to stretch (5,6,16,17). Similar to ST2, interleukin-33 has been suggested to play at least a dual role, acting as a pro-inflammatory cytokine as well as an intracellular nuclear factor with transcriptional regulatory properties (17). In vitro, interleukin-33 antagonizes the effects of angiotensin-II and phenylephrine-mediated activation of nuclear factor kappa beta (NF-κB) in cardiomyocytes, an effect that is antagonized by ST2. Interestingly, treatment with interleukin-33 seems to ameliorate ventricular fibrosis and hypertrophy in mice subjected to ventricular pressure overload, but only in wild-type mice, not those deficient in ST2 (5). Thus, ST2 might act as a soluble decoy receptor for interleukin-33, mitigating the effects of excessive interleukin-33 exposure and therefore mediating the interaction between cardiac myocytes, fibroblasts, and possibly endothelial cells.

A most striking finding of our study was the relationship between ST2 and the risk for mortality at 1 year after presentation with dyspnea. In these subjects, ST2 was at least as potently predictive of death as NT-proBNP, a marker we recently described as having value for predicting death in this population (10). Importantly, even in those with high NT-proBNP levels, we found that the majority of mortality events were primarily concentrated among subjects with elevated ST2 levels at presentation. We also showed elevated ST2 concentrations, although potentially expected in those with acute HF, to be predictive of mortality in those without acute HF as well. It is presently well established that activation of the natriuretic peptide system might be prognostically meaningful among those with critical illness, even in the absence of elevated cardiac filling pressures (18), and might also be useful for predicting death in dyspneic patients without HF (10). In analogy, ST2 levels might be elevated in proportion to severity of critical illness in those with sepsis and trauma (19) and malignancy (20) as well as with exacerbation of severe pulmonary disease (21–25). Indeed, the diagnoses most frequently seen in those decedents with low NT-proBNP but elevated ST2 values were of pulmonary disease origin.

Because both ST2 and NT-proBNP were independent predictors of death, we also showed that the elevation of both markers was associated with the highest rates of death at 1 year in the entire patient cohort as well as in acute HF. Such a multimarker approach for risk stratification has been proposed for patients with acute coronary syndromes (26); our data suggest potential utility for such a multi-marker evaluation of the dyspneic patient, including those with HF.

Limitations of our study include the fact that the biologic role of ST2 in the heart remained poorly understood until recently; with new advances in the understanding of the interleukin-33/ST2 interaction in the heart (5,6), this limitation will be further addressed. Our data are novel inasmuch as they examine a population of acutely symptomatic patients with and without acutely destabilized HF; it is worth noting, however, that in a prior clinical study of patients with chronic HF, serial ST2 concentrations seemed useful for prediction of hazard (7). Our data represent the experience of a single-center study, although the characteristics of our subjects are comparable to multicenter studies of acute dyspnea (27). Lastly, many significant differences between decedents and survivors are identified, which might lead to the risk of spectrum bias and a consequent risk for overestimation of the value of ST2 measurement.

In conclusion, we have demonstrated important associations between the novel marker ST2 and risk for mortality after presentation with acute dyspnea. Future studies are necessary to elucidate potential role(s) for ST2 in the evaluation and management of those with HF or pulmonary diseases.
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