Identification of Serum Soluble ST2 Receptor as a Novel Heart Failure Biomarker

Ellen O. Weinberg, PhD; Masahisa Shimpo, MD, PhD; Shelley Hurwitz, PhD; Shin-ichi Tominaga, MD, PhD; Jean-Lucien Rouleau, MD; Richard T. Lee, MD

Background—Using genomic technology, we previously identified an interleukin-1 receptor family member, ST2, as a gene markedly induced by mechanical strain in cardiac myocytes. The soluble receptor form of ST2 is secreted and detectable in human serum. This study tested the hypothesis that soluble ST2 levels in the serum of patients with severe chronic heart failure are increased in patients with neurohormonal activation.

Methods and Results—Serum samples, clinical variables, and neurohormone levels from the PRAISE-2 heart failure trial (NYHA functional class III-IV; end point, mortality or transplantation) were analyzed. ST2 serum measurements were performed with ELISA on samples from 161 patients obtained at trial enrollment and from 139 of the same patients obtained 2 weeks after trial enrollment. Baseline ST2 levels were correlated with baseline B-type natriuretic peptide (BNP) levels ($r=0.36$, $P<0.0001$), baseline proatrial natriuretic peptide (ProANP) levels ($r=0.36$, $P<0.0001$), and baseline norepinephrine levels ($r=0.39$, $P<0.0001$). The change in ST2 was significant as a univariate predictor of subsequent mortality or transplantation ($P=0.048$), as was baseline BNP ($P<0.0001$) and baseline ProANP ($P<0.0001$). In multivariate models including BNP and ProANP, the change in ST2 remained significant as a predictor of mortality or transplantation independent of BNP and ProANP.

Conclusions—Serum soluble ST2 is a novel biomarker for neurohormonal activation in patients with heart failure. In patients with severe chronic NYHA class III to IV heart failure, the change in ST2 levels is an independent predictor of subsequent mortality or transplantation. (Circulation. 2003;107:721-726.)

Key Words: heart failure • natriuretic peptides • norepinephrine • immune system

Despite improvements in diagnosis and therapy, congestive heart failure is a major problem in the United States, and the impact of heart failure is growing throughout the world.1,2 Present therapies may improve both symptoms and prognosis, but heart failure remains a progressive disease. Therefore, novel approaches to diagnosis and treatment of heart failure are needed.

A major benefit of the explosion of human genomic information is the discovery of new disease pathways. One strategy to define new disease pathways is through high throughput screening for expression of thousands of genes in diseased tissues or cellular models, a process sometimes called functional genomics.3 With this approach, novel gene targets can be rapidly identified through hybridization to DNA microarrays, but additional validation of novel targets in humans is essential.

We previously identified ST2, an interleukin-1 receptor family member, as a mechanically induced gene in cultured rat cardiomyocytes.4 The protein product of ST2 encodes a membrane receptor of the interleukin-1 receptor family and a truncated soluble receptor that can be detected in human serum.5,6 Soluble ST2 is detected in the serum of patients early after acute myocardial infarction and inversely correlates with ejection fraction.7 These findings suggest that ST2 is rapidly and transiently induced in humans and raise the hypothesis that soluble ST2 is increased chronically in patients with heart failure. In addition, the biomechanical stimulation of ST2 in vitro is similar to the mechanical induction of B-type natriuretic peptide (BNP),7 which is a useful diagnostic and prognostic marker in human heart failure.8–10 Thus, this study was also designed to test the hypothesis that human soluble ST2 levels are increased in heart failure patients with increased BNP levels.

Methods

Study Population
The Prospective Randomized Amlodipine Survival Evaluation 2 (PRAISE-2) study was a multicenter, randomized, double-blinded,
parallel group, placebo-controlled study to evaluate the effects of amiodipine 10 mg/d on survival in patients with congestive heart failure of a nonischemic pathogenesis. The trial consisted of patients recruited from 260 sites in the United States and Canada. The neurohormone substudy consisted of 181 patients recruited from 26 centers participating in the study. Both the PRAISE-2 study and the neurohormonal substudy were approved by the institutional review boards of the participating institutions, and subjects gave informed consent. Patients were eligible if they were at least 18 years of age and had heart failure of a nonischemic pathogenesis, symptoms at rest or on minimal exertion (New York Heart Association functional class III or IV), and a left ventricular ejection fraction <30%. All patients were undergoing treatment with angiotensin converting enzyme (ACE) inhibitors and digoxin for at least 3 months. Patients were excluded if they had a recent or remote history of angina. For measurements of ST2 levels in control subjects, serum samples were obtained from subjects with normal left ventricular systolic function (N=9) referred to the echocardiography laboratory at Brigham and Women’s Hospital for symptoms unrelated to heart failure. Subjects gave informed consent.

Assays for ST2, Neurohormones, and Measurement of Oxidative Stress

Blood samples were evaluated at baseline and 2 weeks. Soluble ST2 was measured with a sandwich double monochromal antibody ELISA method (Medical & Biological Laboratories). In brief, serum samples or standards were incubated in microwells coated with anti-human ST2 antibody. After washing, peroxidase-conjugated anti-human ST2 antibody was added into the microwell and incubated. After another washing, the peroxidase substrate was added and the optical density at 450 nm was determined. Circulating catecholamines (norepinephrine, epinephrine, and dopamine), angiotensin II, natriuretic peptides (proatrial natriuretic peptide [ProANP] and BNP), and an index of oxidative stress (adrenolutin) were measured as previously described.11,12

Statistical Analysis

Neurohormone distributions were positively skewed and were described with the median and 5th and 95th percentile. Mann-Whitney analysis was used for comparison of ST2 levels in heart failure patients versus control subjects. Spearman correlations were used for the magnitude and significance of relationships among continuous variables. Rank biserial correlation was used for relationships between dichotomous and continuous variables. Logistic regression was used to estimate odds ratios and 95% confidence intervals for univariate predictors of end point (mortality or transplantation) after inspecting plots of the proportion with end point as a function of each independent variable. Because it is known that ProANP and BNP independently predict end point, multiple variable models were developed to address specifically whether baseline ST2 and whether ST2 change from baseline to 2 weeks contribute significantly to end point prediction beyond the contribution of either ProANP or BNP. Either ProANP or BNP was required to stay in the model, and potential additional predictors were white race, male sex, idiopathic pathogenesis, age, body mass index, left ventricular ejection fraction, creatinine, norepinephrine, epinephrine, dopamine, angiotensin II, and adrenolutin. The multiple variable reduced model containing either ProANP or BNP was selected by a combination of forward, backward, and stepwise selection procedures with significance criteria on 0.15, along with goodness of fit tests and examination of residuals.13 After the reduced model was selected, either baseline ST2 or ST2 change was added. Interaction was allowed between baseline ST2 or ST2 change and ProANP or BNP. This procedure yielded the maximum sample size for testing the additional contribution of baseline ST2 or ST2 change beyond the contribution of ProANP or BNP in a parsimonious model. Unadjusted and adjusted odds ratios and 95% confidence intervals were reported. Additional analyses conducted with baseline ST2, ST2 change, BNP, and ProANP dichotomized according to the respective medians yielded similar results; results using original variables are presented here.

Results

ST2 Levels in Patients With Severe Heart Failure Versus Control Subjects

Serum ST2 levels were significantly higher in patients with severe heart failure (median [5th to 95th percentile], 0.24 [0.16 to 0.70] ng/mL compared with control subjects (0.14 [0.13 to 0.17] ng/mL; P<0.0001).

Baseline Characteristics

Baseline blood samples and clinical indices from 161 patients were available for the present study. Blood samples obtained at baseline as well as 2 weeks after trial enrollment were available from 139 of these patients. Baseline characteristics for all available patients were similar to those for whom baseline and 2-week blood samples were available (Table 1).

ST2 Levels and Clinical and Neurohormonal Variables

There was no significant difference of baseline ST2 in patients randomized to receive amiodipine versus placebo (P=0.84); similarly, there was no significant difference of change in ST2 (ST2 at week 2 minus ST2 at baseline) in patients randomized to receive amiodipine versus placebo (P=0.55). There was a trend toward higher baseline ST2 levels in patients who reached the end point of mortality or transplantation (N=47; median, 0.256 [0.170 to 0.811]) compared with patients who did not reach the end point (N=109; median, 0.233 [0.154 to 0.651]; P=0.070).

Baseline ST2 was significantly positively correlated with baseline BNP (r=0.36, P<0.0001) and with baseline ProANP (r=0.36, P<0.0001), natriuretic peptides secreted from atrial and ventricular myocardium in heart failure, as well as with baseline norepinephrine (r=0.39, P<0.0001)(Table 2). Scatter plots demonstrating these relations and the skewness of the distributions are shown in the Figure, panels A, B, and C, respectively. The change in ST2 (values at week 2 minus values at baseline) was positively correlated with change in BNP (r=0.21, P=0.01), change in ProANP (r=0.29, P=0.0006), baseline dopamine (r=0.22, P=0.01), and age (r=0.19, P=0.03). The change in ST2 was negatively correlated with baseline norepinephrine (r=−0.21, P=0.003) (Table 2).

ST2 Levels and Race, Sex, and Pathogenesis of Heart Failure

ST2 levels and race, sex, and pathogenesis of heart failure are shown in Table 2. ST2 at baseline was significantly lower in white patients compared with nonwhite patients. Baseline ST2 was similar in female versus male patients (P=0.36) and in patients with idiopathic heart failure versus nonidiopathic heart failure (P=0.48). There was a significantly greater decrease in ST2 for nonwhite patients compared with white patients (−0.178 versus −1.33 ng/mL, P=0.0007), possibly reflecting higher baseline ST2 levels in nonwhite patients. The change in ST2 was not affected by sex (P=0.55) or idiopathic pathogenesis (P=0.19).

Univariate Predictors of End Point

Several variables were tested for their ability to predict end point (mortality or transplantation) (Table 3). Univariate
predictors were change in ST2 ($P=0.048$), baseline BNP ($P<0.0001$), baseline ProANP ($P<0.0001$), norepinephrine ($P=0.056$), dopamine ($P=0.043$), creatinine ($P=0.053$), and age ($P=0.01$). Notably, the change in ST2, but not baseline ST2, was predictive. Change in BNP and change in ProANP were not significant predictors of end point despite the excellent predictive power of baseline BNP and ProANP.

Change in ST2 as a Predictor of End Point in Patients With Severe Heart Failure: Multivariate Models

Baseline ST2 and change in ST2 were analyzed with stepwise multivariate analysis to evaluate their significance as independent predictors of mortality or transplantation (Table 4). Baseline BNP and ProANP were powerful predictors of mortality or transplantation, as previously reported.$^{10,14–16}$ Baseline ST2 was not an independent predictor of mortality or transplantation when baseline BNP or baseline ProANP were included in the model (baseline ST2 with BNP, $P=0.6368$; baseline ST2 with baseline ProANP, $P=0.3306$) (Table 4). However, change in ST2 was a significant independent predictor of mortality or transplantation when either BNP ($P=0.0392$) or ProANP ($P=0.0274$) were in the model. These results suggest that change in ST2 is a significant predictor of mortality or transplantation independent of BNP or ProANP in patients with severe heart failure.

Discussion

We identified ST2 in a genomic screen for novel biomechanical pathways involved in the pathophysiology of heart failure. Among thousands of genes, the ST2 gene was the most highly induced by mechanical strain in cardiac myocytes, a system that models on a cellular level the increase in ventricular stress imposed on the myocardium. We explored the relevance of this in vitro approach in the present study with the demonstration of an association between ST2 levels in the peripheral circulation and neurohormonal activation in patients with severe heart failure.

ST2 was first identified as a serum-responsive gene in fibroblasts that encode a secreted protein.$^{17}$ Subsequently, a membrane-anchored receptor form of ST2 was identified,$^{18}$ consisting of an extracellular domain identical to ST2, a transmembrane domain, and an intracellular domain, all with homology to the interleukin-1 receptor, although ST2 does
not bind interleukin-1. ST2 is expressed by T helper-2 (Th2) but not Th1 cells. No ligand and no function have yet been ascribed to either the soluble or membrane forms of ST2. However, soluble ST2 has recently been shown to bind to macrophages in response to the bacterial toxin lipopolysaccharide, which was accompanied by downregulation of proinflammatory cytokines, interleukin-6, interferon-γ, and tumor necrosis factor-α as well as decreased expression of the innate immunity receptor toll-like receptor-4 (TLR4). This suggests that ST2 may regulate inflammatory signals in heart failure.

The few clinical reports thus far on soluble ST2 suggest an immunomodulatory role in disparate human diseases. ST2 is increased in the serum of asthma patients and in some patients with autoimmune diseases. ST2 levels in pleural effusions, but not serum, are increased in patients with lung cancer, in association with an increase in lymphocytes with Th2-dominance, compared with pleural effusions from patients with tuberculosis, which had local Th1 dominance and no increase in ST2. ST2 expression is increased in situ in breast cancer and is associated with reduced disease progression. These findings suggest that confounding systemic inflammatory/immune diseases have the potential to complicate the predictive value of serum ST2 measurements in heart failure, although at the present time, asthma and some autoimmune diseases are the only diseases other than heart failure in which an increase in serum ST2 has been identified. Asthma and some autoimmune diseases are the only diseases other than heart failure in which an increase in serum ST2 has been identified.

ST2 levels were acutely increased in the serum of patients 1 day after myocardial infarction, positively correlated with creatine kinase levels, and inversely correlated with ejection fraction. Our findings suggest a role for ST2 in cardiovascular disease, both acutely after myocardial infarction and chronically in severe chronic heart failure.

In some patients with heart failure, neurohormonal systems are activated, with potential adverse effects. Serum levels of the sympathetic nervous system hormone, norepinephrine, and the natriuretic hormones, BNP and ANP, are important prognostic markers in heart failure that correlate with clinical outcome and have known pathophysiological function in heart failure. The present study demonstrates significant positive correlations between circulating levels of ST2 and BNP, ProANP, and norepinephrine with linear regression.
heart failure. It is possible that ST2 has immunomodulatory effects that are coordinated with hemodynamic or neurohormonal status in patients with severe heart failure.

The mechanisms of induction and regulation of ST2 expression in severe heart failure are not known. Local release of proinflammatory cytokines from cells in stressed or damaged tissues may activate neighboring cells to produce ST2. In support of this, we have shown that ST2 expression is induced in cardiac myocytes by interleukin-1β and phorbol ester and we have also shown that the human ST2 promoter is responsive to interleukin-1β. ST2 may regulate inflammatory responses by acting directly on macrophages. ST2 has been shown to bind to macrophages and selectively down-regulate the expression of proinflammatory cytokines, which may serve to prevent uncontrolled inflammatory reactions. ST2 released in response to stress or injury can contribute toward the polarization of T helper cells to the Th2 phenotype to produce interleukin-10. In peripheral blood mononuclear cells isolated from patients with chronic heart failure, interleukin-10 markedly reduced the ex vivo release of tumor necrosis factor-α, which is elevated in the serum of patients with advanced heart failure. These scenarios suggest an antiinflammatory role for ST2 in heart failure, but this needs to be rigorously evaluated.

We could not determine the cellular source of elevated serum ST2 levels in the present study. Severe heart failure is a systemic disease, and failing cardiac myocytes may not be the sole source of elevated serum ST2 levels. We speculate that soluble ST2 protein may be released from microenvironments in which a local inflammatory or immune response is generated in response to cellular injury or stress.

We found that the change in ST2 (ST2 levels becoming more positive during 2 weeks) was an independent predictor of mortality or transplantation, as was baseline BNP, baseline ProANP, and norepinephrine. Furthermore, in multivariate models to assess the value of ST2 as a predictor of mortality or transplantation, which included BNP and ProANP, change in ST2 was an independent predictor of mortality or transplantation. That the change in ST2 over a 2-week period was a sensitive measure of disease progression. This hypothesis should be examined in other patient populations and with multiple measurements over longer periods of time.

**Limitations**

It should be noted that we could not evaluate ST2 levels over a range of severities of heart failure, because all patients in this study were NYHA functional class III-IV. Thus, this study was limited to the evaluation of ST2 in patients with severe heart failure of nonischemic pathogenesis, and, therefore, the prognostic significance of ST2 over a range of severities of heart failure as well as in heart failure patients.

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<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P</th>
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<td>100 ng/mL</td>
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<td>Change in ST2, 0.1 ng/mL</td>
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*Change indicates that values at week 2 minus baseline have increased (become more positive).
Conclusions

Our findings indicate that in patients with severe heart failure, the levels of circulating ST2 are associated with neurohormonal and sympathetic activation. Furthermore, an increase in ST2 protein in the peripheral circulation is associated with an increase in mortality or transplantation in patients with severe heart failure. The change in serum levels of ST2 over time can provide prognostic information in patients with severe heart failure independent of plasma BNP and ProANP. The pathophysiological contribution of ST2, its possible immune or inflammatory function, and its interaction with the neurohumoral activation in heart failure remain to be elucidated.

Acknowledgments

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