Plasma Soluble ST₂ Levels Correlate With Disease Severity and Predict Clinical Worsening in Patients With Pulmonary Arterial Hypertension

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ABSTRACT

Background: Soluble suppression of tumorigenicity (sST₂) has been proposed to be a marker for biomechanical strain and a possible predictor of mortality in patients with chronic heart failure. The use of sST₂ in pulmonary arterial hypertension (PAH) has not been well defined.

Hypothesis: Plasma sST₂ levels may correlate with the disease severity and predict clinical worsening in PAH.

Methods: We performed a cohort study of 40 idiopathic PAH patients with data on demographics, exercise capacity, echocardiographic parameters, laboratory tests, hemodynamics, and medications. Plasma sST₂ was assessed with the high-sensitivity ST₂ ELISA kit at diagnostic catheterization. All patients were followed up from the date of blood sampling. The endpoint was clinical worsening.

Results: sST₂ was significantly elevated in patients with idiopathic PAH compared with control subjects (28.9 ± 13.9 vs 20.7 ± 7.5 ng/mL, $P = 0.003$). Pearson correlation analysis revealed that sST₂ levels correlated with cardiac index ($r = -0.534$, $P = 0.000$) and pulmonary vascular resistance ($r = 0.350$, $P = 0.027$), and could reflect disease severity of PAH. After a mean follow-up of 14 ± 5 months, 12 patients showed clinical worsening. Receiver operating characteristic analysis suggested that sST₂ levels >31.4 ng/mL discriminated clinical worsening with a sensitivity and specificity of 83.3% and 78.6%, respectively. Kaplan-Meier analysis showed that higher sST₂ levels (>31.4 ng/mL) were associated with poor clinical outcomes ($P = 0.008$). Multivariate Cox regression analysis showed that sST₂ was an independent predictor of clinical worsening (hazard ratio: 6.067, 95% confidence interval: 1.317–27.948, $P = 0.021$).

Conclusions: sST₂ correlates with disease severity and is a significant predictor of clinical worsening in patients with PAH.

Introduction

Pulmonary arterial hypertension (PAH) is a devastating, progressive disease characterized by increasingly elevated pulmonary vascular resistance (PVR), which eventually leads to right-heart failure or premature death.¹ Routine evaluation of PAH is based on exercise capacity, echocardiography, and right-heart catheterization.^{2,3} In recent years, biomarkers such as N-terminal pro-brain natriuretic peptide (NT-proBNP) have emerged as attractive noninvasive tools for assessment and monitoring of PAH severity.^{4–8} These biomarkers can reflect disease severity, predict survival, and be helpful in early identification of patients at risk. However, given the complexity of this disease, it is unlikely that a

single biomarker will be adequate. Therefore, the search for new biomarkers has great clinical significance.

Plasma soluble suppression of tumorigenicity (sST₂), a member of the interleukin-1 (IL-1) receptor family secreted by cardiomyocytes and fibroblasts in response to mechanical strain, has been used as a novel biomarker of myocardial fibrosis and remodeling in many cardiovascular diseases.^{9,10} Expression of sST₂ is markedly up-regulated as early as 1 hour following mechanical strain in cultured myocytes, and 12 hours after presentation for acute myocardial infarction in patients.^{11,12} sST₂ levels correlate with postinfarct remodeling¹³ and independently predict 30-day mortality and heart failure in acute myocardial infarction.¹⁴ In patients with chronic and acutely decompensated heart failure, serum sST₂ levels are increased and strongly associated with both disease severity and mortality.^{15,16} Moreover, among dyspneic patients, sST₂ concentrations are associated with cardiac structure and

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function on echocardiography, and high sST2 concentrations predict long-term mortality.¹⁷ These results indicate that sST2 is a powerful and reliable prognostic biomarker in cardiovascular diseases.

However, current studies mainly concentrate on the value of sST2 in left-heart failure. Studies of sST2 in right-heart failure induced by PAH have been less clear. In outpatients referred for echocardiography, sST2 levels reflected right-side heart size and function and were independent predictors of 1-year mortality.^{18,19} In patients with childhood PAH, Chida et al²⁰ found that the sST2 and NT-proBNP combination was a useful biomarker to predict clinical condition and outcome. Recently, Carlomagno et al²¹ found that serum sST2 was increased in patients with PAH and correlated with right ventricular (RV) dilatation and systolic dysfunction. This study first suggested that sST2 might be a candidate biomarker in the evaluation of PAH. However, as the author mentioned, it was limited by the relatively small number of patients and lack of prognostic assessment. Therefore, with a more widely used commercial enzyme-linked immunosorbent assay (ELISA) kit (Presage ST2 Assay; Critical Diagnostics, San Diego, CA),²² we performed a new study with more PAH patients, aiming to investigate the plasma levels of sST2 in PAH and their possible correlation with clinical outcomes.

Methods

Study Population

Forty patients with idiopathic PAH were prospectively enrolled at Fuwai Hospital from January 2012 to May 2013. The diagnosis of idiopathic PAH was established according to guidelines.¹ Patients with ≥ 1 of the following conditions were excluded: (1) other types of pulmonary hypertension; (2) significant left-ventricular diseases, such as coronary artery disease; (3) acute heart failure; (4) chronic respiratory disorders; (5) connective-tissue diseases; (6) diabetes mellitus; (7) prior targeted therapy; and (8) other diseases, including chronic inflammatory processes or renal or hepatic failure. The following data were gathered: basic demographics, echocardiographic parameters, laboratory tests, medications, hemodynamic measurements from the right-heart catheterization, and exercise tolerance as assessed by 6-minute walk distance (6MWD) and World Health Organization (WHO) functional classification. All patients were followed up by outpatient clinic interview or telephone contact from the date of blood sampling. The composite endpoint was clinical worsening, which was defined as any of 5 different endpoints: death, lung transplantation, hospitalization for PAH, the initiation of a new therapy, or worsening WHO functional class.^{23,24} Data were collected until the latest follow-up in December 2013.

Twenty-four control subjects were selected from a cohort of healthy volunteers. The mean age of control subjects was 30.4 ± 7.5 years, and the ratio of women to men was 19/24 (79%). This study complied with the Declaration of Helsinki and was approved by the institutional review board of Fuwai Hospital.

Hemodynamic Evaluation

Right-heart catheterization was performed according to standard procedures without sedation. In brief, it was

performed using a Swan-Ganz catheter via the right internal jugular to measure mean right atrial pressure (mRAP), mean pulmonary arterial pressure (mPAP), and pulmonary capillary wedge pressure (PCWP). Pulmonary blood flow was determined by the Fick method.²⁵ Pulmonary vascular resistance was calculated by the following equation: $PVR = (mPAP - PCWP) / \text{pulmonary blood flow}$. Cardiac index was calculated by pulmonary blood flow divided by body surface area. Pulmonary vasoreactivity testing was performed as previously described.²⁶ A positive acute response was defined as a decrease in mPAP by ≥ 10 mm Hg to reach an absolute level of < 40 mm Hg without a reduction in cardiac output.¹

Plasma Samples and Biochemical Measurements

Blood samples were obtained in the nonfasting state. Routine biochemistry was analyzed on fresh samples in the clinical laboratory of Fuwai Hospital. Plasma NT-proBNP and big endothelin-1 (Big ET-1) concentrations were determined by a commercially available assay (Biomedica GmbH, Vienna, Austria). Blood samples for the measurement of sST2 were collected with ethylenediaminetetraacetic (EDTA) anticoagulant tube, kept on ice, and centrifuged within 30 minutes. Plasma samples were stored at -80°C until analyzed. The sST2 analyses were performed using the Presage ST2 Assay at BG Medicine (Waltham, MA) as described by Dieplinger et al.²⁷ This assay has been validated with minimal variation (within-run coefficient of variation $< 2.5\%$ and total coefficient of variation $< 4.0\%$) across the wide measurement range of the assay. The biochemical analyses were performed blinded to clinical data and treatment allocation.

Statistical Analysis

The data were described using mean (SD) for continuous variables and number (%) for categorical variables. Statistical differences between the 2 groups were analyzed using an independent Student *t* test and the χ^2 or Fisher exact test for normally distributed variables. The Pearson correlation method was performed to determine the correlation coefficients between sST2 and other variables. The optimal cutoff value of the baseline sST2 was determined by receiver operating characteristic (ROC) analysis. Survival curves were derived using the Kaplan-Meier method and were compared with the log-rank test. A univariate Cox proportional hazards regression model was used to select potential predictors of clinical worsening. Multivariate Cox proportional hazards regression with backward elimination was used to identify independent predictors of clinical worsening. Statistical analysis was performed by SPSS version 16.0 (SPSS Inc., Chicago, IL). $P < 0.05$ was considered statistically significant.

Results

Overall Characteristics of Study Population

Forty patients with idiopathic PAH and 24 controls were enrolled in the study. No significant difference was found between the 2 groups in age and sex. Baseline demographic, clinical, and hemodynamic features are shown in the Table 1.

Table 1. Baseline Characteristics of Study Population and Correlation Analysis

Characteristic	Overall, N = 40	sST2 ≤31.4 ng/mL, n = 24	sST2 >31.4 ng/mL, n = 16	P Value ^a	Correlation With sST2	
					r	P Value
Age, y	32.0 ± 10.9	32.7 ± 11.0	30.9 ± 11.0	NS	−0.091	NS
Female, n (%)	34 (85)	22 (92)	12 (75)	NS	—	—
WHO class III–IV (%)	21 (52.5)	13 (54)	8 (50)	NS	—	—
6MWD, m	418.2 ± 88.2	406.7 ± 94.9	435.4 ± 76.8	NS	0.040	NS
Cr, μmol/L	66.0 ± 14.6	63.1 ± 12.5	70.4 ± 16.7	NS	0.284	NS
NT-proBNP, pg/ml	1580.8 ± 935.5	1477.1 ± 839.6	1736.4 ± 1073.1	NS	0.221	NS
Uric acid, μmol/L	442.0 ± 114.3	415.0 ± 99.0	482.3 ± 126.6	NS	0.383	0.015
Big ET-1, fmol/mL	0.74 ± 0.57	0.59 ± 0.50	0.95 ± 0.62	0.048	0.385	0.014
LVEDd, mm	36.1 ± 4.3	36.5 ± 4.6	35.4 ± 4.0	NS	−0.034	NS
RVEDd, mm	33.5 ± 6.1	32.4 ± 6.0	35.0 ± 6.2	NS	0.321	0.043
Arterial oxygen saturation, %	95.5 ± 3.0	95.9 ± 2.1	94.9 ± 3.9	NS	−0.381	0.015
ScvO ₂ , %	69.8 ± 7.3	72.2 ± 5.2	66.2 ± 8.5	0.008	−0.592	0.000
mRAP, mm Hg	4.9 ± 4.7	3.9 ± 3.2	6.4 ± 6.1	NS	0.511	0.001
Cardiac index, L/min/m ²	2.6 ± 0.7	2.9 ± 0.6	2.2 ± 0.5	0.001	−0.534	0.000
mPAP, mm Hg	60.9 ± 17.6	56.6 ± 18.9	67.4 ± 13.6	NS	0.206	NS
PVR, Wood units	14.3 ± 6.3	11.9 ± 7.0	16.7 ± 3.7	0.016	0.350	0.027

Abbreviations: 6MWD, 6-minute walking distance; Big ET-1, big endothelin-1; Cr, creatinine; LVEDd, left ventricular end-diastolic dimension; mPAP, mean pulmonary arterial pressure; mRAP, mean right atrial pressure; NS, not significant; NT-proBNP, N-terminal pro-brain natriuretic peptide; PVR, pulmonary vascular resistance; RVEDd, right ventricular end-diastolic dimension; ScvO₂, mixed venous oxygen saturation; SD, standard deviation; sST2, soluble suppression of tumorigenicity; WHO, World Health Organization.
Data are presented as n (%) and mean ± SD.
^aP value for between-group comparisons; *P* < 0.05 was considered statistically significant.

Plasma sST2 levels were significantly higher in patients with idiopathic PAH than in control subjects (28.9 ± 13.9 vs 20.7 ± 7.5 ng/mL, respectively, $P = 0.003$; Figure 1). At the time of venous sampling, no patient was receiving PAH-targeted therapies. After the diagnosis, 33 patients (82.5%) were treated with specific PAH drugs: sildenafil (18 patients), bosentan (13 patients), iloprost (1 patient), and beroprost (1 patient). All patients received single-drug therapy, and no patient received combination therapy. The other 7 patients were treatment-naïve for economic reasons.

Clinical Outcomes

All patients were followed up until December 2013. No patient was lost to follow-up. After a mean follow-up of 14 ± 5 months, 12 patients showed clinical worsening; there were 4 deaths (1 sudden, 3 for right-heart failure), 2 hospitalizations for PAH, 3 requiring addition of other PAH treatments, and 3 increases in WHO functional class. The ROC analysis showed that an sST2 level >31.4 ng/mL was predictive of clinical worsening with a sensitivity and specificity of 83.3% and 78.6%, respectively (area under the curve = 0.789, $P = 0.004$; Figure 2). Ten patients (10/16) with sST2 >31.4 ng/mL showed clinical worsening (positive predictive value, 62.5%) and 22 (22/24) patients with sST2

Figure 1. Plasma levels of sST2 in patients with IPAH and control subjects. Abbreviations: IPAH, idiopathic pulmonary arterial hypertension; sST2, soluble suppression of tumorigenicity. * $P < 0.05$ vs control.

≤31.4 ng/mL did not worsen (negative predictive value, 91.7%). The event-free survival curves were significantly different between these 2 subgroups (log-rank $P = 0.008$; Figure 3).

Figure 2. Receiver operating characteristics curve. sST2 > 31.4 ng/mL discriminated clinical worsening with a sensitivity of 83.3% and a specificity of 78.6% (area under the curve = 0.789). Abbreviations: CI, confidence interval; sST2, soluble suppression of tumorigenicity.

Figure 3. Event-free survival curves in patients with sST2 > 31.4 ng/mL (solid line) or ≤ 31.4 ng/mL (dashed line). Higher sST2 levels were associated with poor event-free survival. Abbreviations: sST2, soluble suppression of tumorigenicity.

At univariate analysis using the Cox regression model, cardiac index (cutoff at 2.5 L/min; hazard ratio [HR]: 0.249, 95% confidence interval [CI]: 0.067–0.920, $P = 0.037$), PVR (cutoff at 13.5 Wood units; HR: 3.773, 95% CI: 1.019–13.973, $P = 0.047$), and sST2 (cutoff at 31.4 ng/mL; HR: 6.067, 95% CI: 1.317–27.948, $P = 0.021$) were predictive parameters of clinical worsening. All the cutoff points were identified by ROC analysis. The only independent predictor of clinical worsening, as identified by multivariate Cox proportional hazards regression analysis, was sST2 (HR: 6.067, 95% CI: 1.317–27.948, $P = 0.021$).

Soluble Suppression of Tumorigenicity and Pulmonary Arterial Hypertension Severity

Baseline patient characteristics, stratified according to sST2 cutoff value (31.4 ng/mL), are presented in the Table 1. Patients with the high sST2 levels showed much higher disease severity, including lower cardiac index and mixed venous oxygen saturation (ScvO₂), and higher PVR and Big ET-1. Pearson correlation analysis revealed that plasma sST2 levels correlated positively with mRAP ($r = 0.511$, $P = 0.001$), PVR ($r = 0.350$, $P = 0.027$), uric acid ($r = 0.383$, $P = 0.015$), Big ET-1 ($r = 0.385$, $P = 0.014$), and RV end-diastolic dimension ($r = 0.321$, $P = 0.043$). In contrast, plasma sST2 levels correlated negatively with ScvO₂ ($r = -0.592$, $P = 0.000$), cardiac index ($r = -0.534$, $P = 0.000$), and arterial oxygen saturation ($r = -0.381$, $P = 0.015$; Table 1). There were no significant correlations between plasma sST2 levels and WHO class, 6MWD, and mPAP.

Discussion

ST2 is an IL-1 receptor family member that is expressed both in a transmembrane receptor isoform (ST2L) and a soluble isoform (sST2). The ligand of ST2 is IL-33, which is involved in reducing fibrosis and hypertrophy in the myocardium. The IL-33/ST2 signal is directly regulated by mechanical strain of cardiac muscle and its activation has been shown to be cardioprotective through reducing left-ventricular hypertrophy in response to pressure.^{9,28} sST2, acting as a “decoy” receptor for IL-33, can bind circulating IL-33 and dampen its protective effects, leading to abnormal cardiac remodeling and death.^{10,29} sST2 has emerged as a new biomarker for disease severity and prognosis in heart failure. In this study, we specially extended the value of sST2 in PAH, which is a common cause of right-heart failure. We found that plasma sST2 levels were elevated in idiopathic PAH patients, and sST2 levels correlated with hemodynamic parameters and other biomarkers, including uric acid and Big E-1. Our results also suggested that the elevation of sST2 levels was associated with worse clinical outcomes.

In our study, we used a high-sensitivity sST2 ELISA kit (Presage ST2 Assay), which was approved by the US Food and Drug Administration and has been used in assessing the prognosis of patients with chronic heart failure.²² Our results were consistent with previous studies with another ELISA kit from R&D systems.²¹ During PAH progression, elevated PVR will lead to the deterioration of RV function, which transitioned from compensated hypertrophy to dilatation and failure.³⁰ Experimental data have suggested that mechanical strain could induce the expression of both IL-33 and sST2 in cardiomyocytes and fibroblasts.⁹ In our study, sST2 is correlated with RV end-diastolic dimension, which can reflect the dilatation of the right ventricle. Therefore, increased sST2 levels in PAH may be due to mechanical strain-mediated local myocardial release. However, we cannot exclude extramyocardial sources of sST2, which include endothelium and leukocytes.³¹ Moreover, previous studies have shown that the IL-33/ST2 signal may be implicated in the pathogenesis of pulmonary vascular remodeling.^{32,33} Hence, whether ST2 increase

directly depends on pulmonary vascular remodeling will need further investigation.

In this study, we found that sST2 correlated with cardiac index and PVR and could reflect disease severity in idiopathic PAH. Carlomagno et al²¹ found that sST2 correlated with parameters of RV structure and function measured by magnetic resonance imaging in PAH patients. Among dyspneic patients, sST2 concentrations were related to RV function and could predict long-term mortality.¹⁷ Agoston-Coldea et al¹⁹ also demonstrated that sST2 levels were correlated with and highly predictive of RV dysfunction in patients with secondary pulmonary hypertension owing to chronic obstructive pulmonary disease. From a hemodynamic perspective, we concluded that patients with a poorer RV function might have higher ventricular filling pressures and transmural wall stress, thereby increasing myocardial stretch and sST2 release. Hence, sST2 may be a good biomarker in reflecting RV function.

As RV function is an important determinant of patient outcome,³⁰ we suppose that sST2 may also have great value in predicting prognosis in patients with PAH. Clinical worsening is a composite endpoint that can truly reflect the clinical status and degree of disease progression. It has been used as the primary endpoint of morbidity and mortality in several recently published studies.³⁴ In this study, we found that the elevation of sST2 levels could predict clinical worsening in idiopathic PAH patients. A recently published study by Chida et al also suggested that elevated sST2 and NT-proBNP levels could predicted poor outcome in patients with childhood idiopathic PAH.²⁰ These findings demonstrate that sST2 is a novel indicator of prognosis in PAH and can be used in combination with conventional biomarkers.

There are several limitations to our study. First, it is limited by a relatively small number of patients with a small number of clinical events. Only idiopathic PAH patients were enrolled, and other types of PAH were not included. Second, sST2 levels were only detected at baseline; the effect of treatment on sST2 expression was unknown. It was not clear whether sST2 could be used to evaluate the therapeutic effects. Third, we did not measure levels of IL-33, which might offer additional mechanistic insight into our observed associations. In addition, magnetic resonance imaging was not performed to evaluate RV structure and function in this study. Therefore, further studies with a large sample size and precision method are still needed to identify sST2 as a novel biomarker in PAH.

Conclusion

Plasma sST2 levels are increased in patients with PAH, and the elevation of sST2 levels is correlated with PAH severity and can predict clinical worsening. Our findings suggest that sST2 may be a novel prognostic biomarker in PAH.

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