Original Research

Soluble form of suppression of tumorigenicity-2 predicts clinical stability of inpatients with community-acquired pneumonia

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Impact statement

Our study demonstrated the predictive value of sST2 in community-acquired pneumonia, providing a new perspective for evaluating patients with CAP in clinical practice. In our research, we found that sST2, an established biomarker of acute/choric heart failure, was significantly correlated with the severity and prognosis of CAP, which made it a potential early monitoring index for CAP. Moreover, we respectively combined sST2 with the PSI score or the CURB-65 scores, finding more accurate models for prognosis evaluation and risk classification of CAP, which we believe in being a potentially powerful tool for clinicians to provide appropriate clinical interventions to CAP patients early.

Abstract

The soluble form of the suppression of tumorigenicity-2 (sST2) is a biomarker for risk classification and prognosis of heart failure, and its production and secretion in the alveolar epithelium are significantly correlated with the inflammation-inducing in pulmonary diseases. However, the predictive value of sST2 in pulmonary disease had not been widely studied. This study investigated the potential value in prognosis and risk classification of sST2 in patients with community-acquired pneumonia. Clinical data of ninety-three CAP inpatients were retrieved and their sST2 and other clinical indices were studied. Cox regression models were constructed to probe the sST2's predictive value for patients' restoring clinical stability and its additive effect on pneumonia severity index and CURB-65 scores. Patients who did not reach clinical stability within the defined time (30 days from hospitalization) have had significantly higher levels of sST2 at admission ($P < 0.05$). In univariate and multivariate Cox regression analysis, a high sST2 level ($\geq$72.8 ng/mL) was an independent reverse predictor of clinical stability ($P < 0.05$). The Cox regression model combined with sST2 and CURB-65 (AUC: 0.96) provided a more accurate risk classification than CURB-65 (AUC:0.89) alone (NRI: 1.18, IDI: 0.16, $P < 0.05$). The Cox regression model combined with sST2 and pneumonia severity index (AUC: 0.96) also provided a more accurate risk classification than pneumonia severity index (AUC:0.93) alone (NRI: 0.06; IDI: 0.06, $P < 0.05$). sST2 at admission can be used as an independent early prognostic indicator for CAP patients. Moreover, it can improve the predictive power of CURB-65 and pneumonia severity index score.

Keywords: Tumorigenicity-2, pneumonia, CURB-65, prognosis, classification, pneumonia severity index

***Experimental Biology and Medicine* 2021; 246: 2297–2306. DOI: 10.1177/15353702211027116**

Introduction

Community-acquired pneumonia (CAP) is a common disorder that is potentially life threatening, especially in older adults and those with comorbid disease, responsible for great morbidity and high costs..[1,2] A study demonstrated that among the inpatients diagnosed with CAP, 21% required intensive care in ICU, 6% required invasive mechanical ventilation, and 2% eventually deceased.[3] Early risk classification and prognostic evaluation are clinically important to CAP patients and are determining factors of the selection of clinical interventions. Accurate prediction and precise quantification of the probability of serious adverse events (death, serious complications) allow clinicians to obtain advanced knowledge about the outcome expectations of the patients and are critical for the decision of initial management, such as the intensity of the patients' care, the scale of the diagnostic laboratory testing, and the application of antibiotics. However, till now

the clinical application of index-based prognosis is still very limited, mainly due to the considerable variances in the underlying pathogen and in the crowds' sensitivities and resistances to CAP. [4] Therefore, we urgently need an accurate and convenient method to evaluate CAP.

A variety of inflammatory biomarkers, such as C-reactive protein (CRP), procalcitonin (PCT), and serum amyloid A (SAA), have been widely used in CAP diagnosis, risk classification, and monitoring.[1,5] Interleukin (IL)-33, as a member of the IL-1 cytokine family, mediates tissue inflammation and repair response in many organs, such as the lung, skin, kidneys, central nervous system, and cardiovascular and gastrointestinal systems.[6] Suppression of tumorigenicity-2 (ST2) is an important receptor of IL-33 and a member of the toll-like/interleukin (IL)-1 receptor superfamily. ST2 is secreted by hematopoietic cells and epithelial cells that mediates the Th2 response.[7,8] ST2 has mainly two isoforms: Soluble (sST2) and transmembrane (ST2L) isoforms.[9,10]

IL-33 combination with ST2L on the tumor, immune, and myocardial cells, can activate mitogen-activated protein kinase (MAPK) and nuclear factor (NF-kB) signaling, resulting in the activation and regulation of Th2 cellular immune response, and the inflammatory cascade.[11] The ST2L-IL-33 signaling pathway has been found to play an important role in heart diseases.[12,13] As a decoy receptor, sST2 can inhibit the activity of the ST2L-IL-33 signaling pathway by competitive combination with IL-33.

Since recent decades, sST2 has been utilized as a biomarker for many diseases. In 2013, due to its high sensitivity and specificity, sST2 was recommended by the ACCF/AHA Guideline for the Management of Heart Failure for the prediction of in-hospital and long-term mortality of heart failure patients.[14] Moreover, studies found that the level of sST2 is significantly elevated in active inflammatory bowel disease, acute cardiac and small bowel transplant allograft rejection, colon and gastric cancers, gut mucosal damage during viral infection, pulmonary disease, heart disease, and graft-versus-host disease.[15–18] Recently, alveolar epithelial and bronchial cells were found to be one of sources of sST2 secretion,[19] and the level of sST2 in peripheral blood is significantly elevated in patients with inflammatory diseases of the lower respiratory tract, such as asthma and chronic obstructive pulmonary disease (COPD).[20] Nevertheless, the potential of sST2 as a CAP biomarker has not been specifically investigated.

In this study, we aimed at investigating the correlation between the level of sST2 at admission and CAP severity, evaluating its potential to be a predictive indicator for patients' in-hospital prognosis. In addition, we evaluated whether sST2 could improve predictive powers of the CURB-65 score and pneumonia severity index (PSI), respectively.

Materials and methods

Study design

This retrospective study was approved by the ethics committee of The First Affiliated Hospital of Guangzhou Medical University (Reference number: GYFYY-2016–73). The electronic medical record system was searched for patients who met our recruitment criteria. Written consent forms were obtained from the subjects and their clinical data were reproduced for the next step investigation. Patients were divided into two groups based on whether they have achieved the study endpoint of clinical stability: group A (achieving the endpoint of clinical stability) and group B (not achieving the endpoint of clinical stability); and predictive value of sST2 was probed based on the observed differences between these two groups.

Patient enrollment

Ninety-three CAP patients hospitalized and 33 healthy controls between 2019 and 2020 were identified as qualified subjects for our investigation. All the patients were diagnosed with CAP according to the current community-acquired pneumonia treatment recommendation.[21] CAP was diagnosed in the presence of a new infiltrate on the chest radiograph and at least one of the following acute respiratory signs and symptoms: cough, sputum production, dyspnea, the core body temperature of 38°C or higher, auscultatory findings of abnormal breathing sounds or rales, leucocyte count higher than 10×10^9/L or less than 4×10^9/L. The exclusion criteria were: (1) heart disease, including hypertensive heart disease, ischemic heart disease, valvular heart disease, heart failure, arrhythmia, pulmonary heart disease, myocardiopathy, and congenital heart disease; (2) hospital-acquired pneumonia; (3) age <20 years; (4) lack of clinical record for CURB-65 and PSI assessment; and (5) severe immunosuppression defined as one of the following: infection with human immunodeficiency virus and a CD4 cell count that is below 350 cells per μL, immunosuppressive therapy after solid organ transplantation.

Measurement of sST2 and the retrieving of clinical data

Venous blood samples of patients at admission (0–24 h) were collected. Whole blood samples were centrifuged at 3000*g* for 15 min at room temperature, then sera were aliquoted and stored at −80°C upon analysis. The serum samples were prepared by the sST2 detection kit (ST22003001) and then the sST2 levels were detected by the Jet-iStar 3000 (Joinstar Biomedical Technology Co., LTD, China) according to the manufacturer's instruction. The primary antibody was anti-sST2 mouse monoclonal antibody, and the secondary antibody was carboxyfluorescein diacetate N-succinimidyl ester (CFSE)-labeled anti-sST2 mouse monoclonal antibody. The absorbance of carboxyfluorescein diacetate N-succinimidyl ester (CFSE)-labeled anti-sST2 mouse monoclonal antibody-sST2- anti-sST2 mouse monoclonal antibody complex was measured at 635 mm. Finally, the quantified sST2 levels were obtained by a standard curve drawn from the standard concentration solutions measured on the same plate.

Definition and determination of the study endpoint

To evaluate patients' ability to rehabilitate, we set the study endpoint as the patients' restoring of clinical stability. The diagnosis of clinical stability was made following the current CAP guidelines of American Thoracic Society (ATS) and the study of Claudine Angela Blum et al.[21,22] The time to clinical stability was defined as the days from admission to the date when the patient has stable vital signs for at least 24 h. Stable vital signs were defined as a temperature of 37.8°C or lower, a heart rate of 100 beats per min or lower, a spontaneous respiratory rate of 24 beats per min or lower, systolic blood pressure ≥90 beats per min and without vasopressin (patients with hypertension ≥100 beats per min), a normal mental state, the ability for oral intake, and a partial pressure of oxygen in artery (PaO2) ≥60 mm Hg or pulse oximetry ≥90%. Clinical stability was determined only when all the above criteria were met.

Evaluation of PSI and CURB-65 score

Following the guidance of the American Thoracic Society (ATS),[23,24] we evaluated the patients' PSI and CURB-65 score based on their clinical information, including age, sex, complication (tumor, hepatopathy, cerebrovascular disease, and nephropathy), mental status, respiratory rate, systolic/diastolic blood pressure, temperature, pulse, pH, blood urea nitrogen, serum sodium, blood glucose, hematocrit (HCT), PaO2, hydrothorax. Patients with a pneumonia severity index score of 1–3 were defined as low-risk, 4 as intermediate-risk, and 5 as high-risk patients. Patients with a CURB-65 score of 0–1 were defined as low-risk patients, 2 as intermediate-risk, and 3–5 as high-risk patients.

Patient characteristics

A total of 251 CAP patients and 33 healthy controls were eligible for the study. Among the 251 CAP patients, 26 were excluded due to the absence of informed consent, 24 were excluded due to their age (age <20 years). In the 201 initially enrolled patients, 39 patients were excluded due to hospital-acquired pneumonia, 33 due to the lack of clinical information for PSI and CURB-65 evaluation, 36 due to severe immunosuppression condition (13 with lung transplantation, 12 with acquired immune deficiency syndrome, and 11 with active tuberculosis). Finally, 93 CAP patients and 33 healthy controls were enrolled (Supplementary Figure 1) and their baseline characteristics are shown in Table 1. The 93 CAP patients had higher heparin-binding protein (HBP), N-terminal pro-brain natriuretic peptide (NT-proBNP), PCT, CRP, SAA, blood glucose, lymphocyte count, D-dimer, and oxygenation index (PaO2/FiO2) than healthy controls. However, the differences in interleukin-6 (IL-6), leukocyte count, blood urea nitrogen (BUN), and

Table 1. Patient characteristics.

	Healthy controls ($n = 33$)	All patients ($n = 93$)	Clinical stability ($n = 79$)	Non-clinical stability ($n = 14$)	*P*$ value	*P** value
Sex (M/F)	20/13	53/40	44/35	9/5	0.876	0.760
Age (IQR)	51 (46.00–64.00)	6200 (48.50–67.00)	59.00 (41.00–66.00)	65.50 (52.00–70.25)	0.120	0.026
PSI Score						<0.001
1 (*n*)	0	21	21	0		
2 (*n*)	0	27	27	0		
3 (*n*)	0	15	15	0		
4 (*n*)	0	21	14	7		
5 (*n*)	0	9	2	7		
Curb-65 Score						<0.001
Low risk (0–1) (*n*)	0	60	58	2		
Moderate risk (2) (*n*)	0	16	14	2		
High risk (3–5) (*n*)	0	17	7	10		
Laboratory tests						
HBP (ng/mL)	13.36 (11.67–22.26)	21.68 (13.23–42.78)	22.12 (13.27–44.19)	19.32 (12.44–38.69)	0.014	0.583
IL-6 (pg/mL)	25.50 (14.05–31.80)	27.75 (15.35–40.65)	27.60 (14.75–39.03)	28.25 (15.50–89.50)	0.337	0.597
NT-proBNP (pg/mL)	73.20 (63.80–129.80)	125.90 (75.30–320.30)	105.10 (73.20–201.00)	1057.80 (588.65–2008.60)	0.039	<0.001
PCT (ng/mL)	0.09 (0.09–0.12)	0.09 (0.09–0.28)	0.09 (0.09–0.16)	1.04 (0.175–6.64)	0.035	<0.001
sST2 (ng/mL)	7.10 (4.30–9.60)	16.00 (7.95–72.80)	14.00 (7.50–36.30)	173.95 (107.75–201.00)	<0.001	<0.001
CRP (ng/mL)	1.00 (0.49–2.69)	6.60 (1.35–35.90)	3.98 (1.10–21.19)	63.53 (19.34–98.18)	0.001	0.001
SAA (ng/mL)	4.90 (4.90–7.15)	10.70 (4.90–182.85)	8.30 (4.90–112.50)	201.00 (66.85–201.00)	0.001	0.003
Blood glucose (mmol/L)	4.80 (4.30–5.19)	5.09 (4.44–6.93)	4.93 (4.43–5.60)	11.03 (7.82–16.78)	0.032	<0.001
BUN (mmol/L)	4.70 (4.20–5.90)	5.30 (4.23–7.55)	5.10 (4.20–6.50)	14.10 (9.25–18.00)	0.226	<0.001
Leukocyte ($\times 10^9$/L)	7.70 (6.10–8.70)	7.85 (5.95–10.33)	7.90 (6.00–10.15)	7.50 (5.30–13.30)	0.335	0.931
Neutrophils ($\times 10^9$/L)	4.40 (3.30–5.75)	5.05 (3.40–7.65)	4.80 (3.35–7.35)	5.40 (4.50–12.60)	0.074	0.065
Lymphocyte ($\times 10^9$/L)	2.3 (1.55–2.45)	1.50 (0.90–2.20)	1.70 (1.05–2.35)	0.40 (0.25–1.05)	0.003	<0.001
Oxygenation index (mmHg)	462.14 (411.90–517.62)	413.00 (319.00–485.00)	424.00 (374.25–488.75)	219.50 (137.75–324.50)	0.01	0.002
D-dimer (ng/mL)	133.10 (100.50–248.60)	388.10 (147.00–940.78)	296.20 (134.13–606.00)	1932.85 (1181.60–6052.95)	<0.001	<0.001

P$ value: *P* value between healthy controls and all patients; *P** value: *P* value between patients reaching clinical stability and patients not reaching clinical stability; M: male; F: female; PSI: pneumonia severity index; HBP: heparin-binding protein; IL-6: interleukin-6; NT-proBNP: N-terminal pro-brain natriuretic peptide; PCT: procalcitonin; sST2: soluble growth stimulation expressed gene 2; CRP: C-reactive protein; SAA: serum amyloid A; BUN: blood urea nitrogen; oxygenation index, PaO_2/FiO_2.

neutrophil count between CAP patients and healthy controls were not significant.

Statistical analysis

The Shapiro–Wilk test was used to evaluate the normality of continuous data. All continuous data were non-normal distribution. Continuous data were shown as median (interquartile range). Median values of metric variables were compared among the groups using the Mann–Whitney–Wilcoxon rank sum test, or in case of comparison of three or more groups, the Kruskal–Wallis test was used. Categorical data were compared using a Chi-square test or Fisher's exact test. Spearman's correlation coefficient was used for all data correlation analysis. Cox-regression analysis was used to calculate hazard ratios (HR) and to establish multivariate COX regression models. The receiver operating characteristic (ROC) curve analysis was used to calculate the area under the curve (AUC) and the cut-off values (determined by Youden indexes). Using AUC, net reclassification improvement (NRI)[25] and integrated discrimination improvement (IDI)[25] compared different competent models. $P < 0.05$ was considered statistically significant. All statistical analyses in this study were performed using SPSS Statistics version 22.0 (IBM, New York, USA) or R language (version 4.0.2, Bell Laboratories).

Results

Statistical analysis of sST2 and other laboratory indices

The results showed that all patients have had significantly higher sST2 levels (16.00 vs. 7.10 ng/mL, $P < 0.05$, Figure 1 (a)) than healthy controls. Then, we studied the difference of sST2 levels between the patients reaching clinical stability (group A) and the patients not reaching clinical stability (group B). Similarly, the results showed that the patients in group B have had significantly higher sST2 levels at admission (137.95 vs. 14.00 ng/mL, $P < 0.05$, Figure 1(b)). After 30 days in-hospital observation, 79 patients reached the study endpoint, whereas 14 patients were not able to reach clinical stability (one patient died due to multiple organs' failure, five referral patients, and nine patients were still under treatment). The patients in group B were older and had higher PSI, CURB-65 scores, NT-proBNP, PCT, CRP, SAA, BUN, lymphocyte count, D-dimer, and oxygenation index (PaO2/FiO2) than group A. However, the differences in sex, HBP, IL-6, leukocyte count, and neutrophil count between patients in the two groups were not significant.

We also collected the information of causative pathogens of 93 CAP patients from the electronic medical record system to evaluate the differences among various pathogens-caused patients. There are six CAP patients with *Acinetobacter baumannii*, two patients with *Escherichia coli*, seven patients with *Klebsiella pneumoniae*, one patient with *Streptococcus pneumoniae*, five patients with *Staphylococcus aureus*, four patients with *Haemophilus influenzae*, one patient with *Legionella pneumophila*, six patients with *Pseudomonas aeruginosa*, 61 patients unknown. The sST2 levels of patients with *Acinetobacter baumannii* were significantly higher than patients unknown ($P = 0.003$). However, sST2 levels did not differ among the patients with clear causative pathogens (Supplementary Table 1).

Correlation between serum sST2 and other clinical indices

The level of sST2 was positively correlated with the PSI level ($r = 0.81$, $P < 0.05$) and CURB-65 level ($r = 0.64$, $P < 0.05$) (Figure 2(a) and (b), respectively). Moreover, when evaluating the correlations between patients' sST2 serum concentration at admission and other laboratory indices (Table 2), we found that sST2 serum concentration was positively correlated with NT-Pro, PCT, CRP, SAA, leukocyte count, neutrophil count, neutrophil ratio, and D-dimer, and was negatively correlated with lymphocyte count/ratio and oxygenation index (all $P < 0.05$). However, there were no correlations between sST2 concentration, HBP, and IL-6.

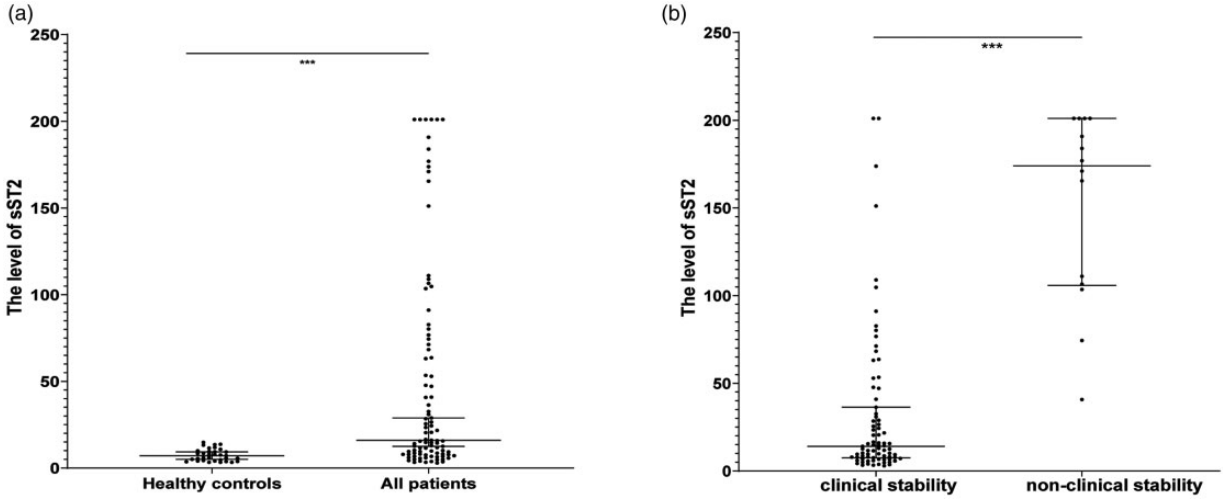

Figure 1. sST2 level in different group. (a) At admission, serum sST2 levels of CAP patients were significantly higher than healthy controls. (b) At admission, serum sST2 levels of CAP patients that did not reach clinical stability, was significantly higher than those in patients who reached clinical stability; ***$P < 0.001$.

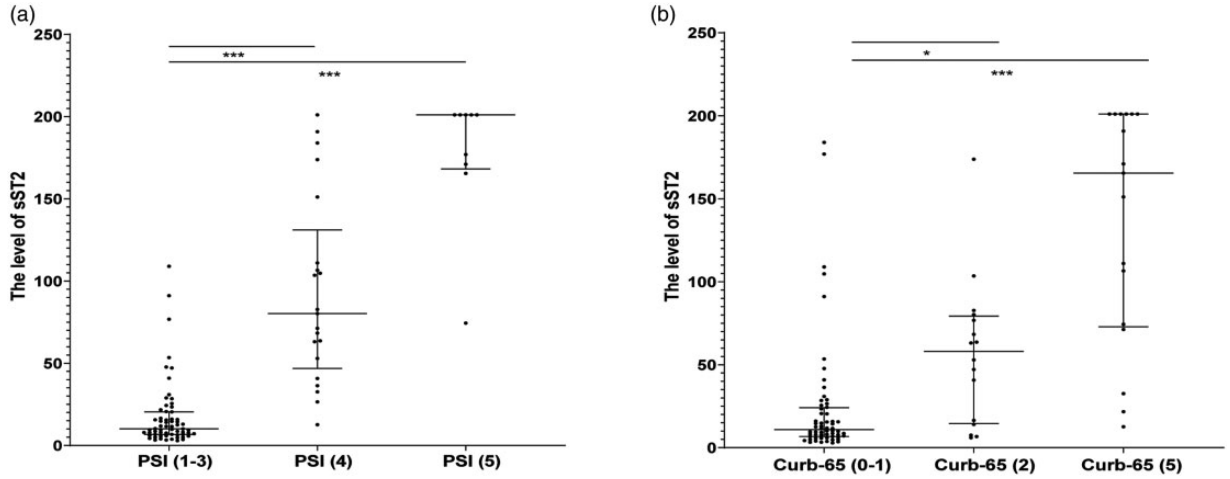

Figure 2. sST2 level in different group of PSI and Curb-65. (a) At admission, serum sST2 levels of CAP patients significantly and positively correlated with the PSI level; (b) At admission, serum sST2 levels of CAP patients significantly and positively correlated with the CURB-65 level. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 2. Correlations between sST2 and laboratory parameters.

	sST2 ($n = 93$)	
	R	*P*
HBP (ng/mL)	0.171	0.103
IL-6 (pg/mL)	0.048	0.660
NT-proBNP (pg/mL)	0.578	<0.001
PCT (ng/mL)	0.619	<0.001
CRP (ng/mL)	0.705	<0.001
SAA (ng/mL)	0.738	<0.001
Blood glucose (mmol/L)	0.539	<0.001
Blood urea nitrogen (mmol/L)	0.601	<0.001
Leukocyte count ($\times 10^9$/L)	0.295	0.005
Neutrophil count ($\times 10^9$/L)	0.493	<0.001
Neutrophil (%)	0.727	<0.001
Lymphocyte count ($\times 10^9$/L)	−0.634	<0.001
Lymphocyte (%)	−0.702	<0.001
Oxygenation index (mmHg)	−0.495	<0.001
D-dimer (ng/mL)	0.508	<0.001

R: correlation coefficient; HBP: heparin-binding protein; IL-6: interleukin-6; NT-proBNP: N-terminal pro-brain natriuretic peptide; PCT: procalcitonin; sST2: soluble growth stimulation expressed gene 2; CRP: C-reactive protein; SAA: serum amyloid A; oxygenation index: PaO_2/FiO_2.

Soluble ST2 as a reverse predictor of clinical stability

We performed ROC curve and Cox regression analyses to assess sST2's predictive value for clinical stability. Using the ROC curve analysis, the AUC of sST2 in predicting patients who did not reach clinical stability was 0.94 ($P < 0.05$), larger than that of CRP (AUC: 0.83, $P < 0.05$) and PCT (AUC: 0.75, $P = 0.01$); and the cut-off value of sST2 evaluated by the Youden index was 72.8 ng/mL (sensitivity: 92.9%, specificity: 87.3%) (Figure 3(a)). The Kaplan–Meier curve analysis showed that patients with high serum sST2 levels (≥72.8 ng/mL) had a lower possibility of reaching clinical stability when compared with patients had low serum sST2 levels (<72.8 ng/mL), with an HR of 0.13 (95% CI: 0.06–0.26) ($P < 0.05$, Figure 3(b)). We then performed univariate Cox regression and multivariate Cox regression analyses to further evaluate the predictive potential of serum sST2 level, CURB-65 score, PSI score, PCT, and CRP. In the univariate Cox regression analysis, high serum sST2 levels (≥72.8 ng/mL), high CURB-65 scores (3–5), high PSI scores (5), PCT and CRP negatively predicted the clinical stability of patients, with HRs (95% CI) of 0.13 (0.06–0.26, $P < 0.05$), 0.15 (0.07–0.33, $P < 0.05$), 0.05 (0.01–0.19, $P < 0.05$), 0.35 (0.18–0.69, $P < 0.05$), and 0.98 (0.97–0.99, $P < 0.05$), respectively. In the multivariate Cox regression analysis, high serum sST2 levels (≥72.8 ng/mL), high PSI scores (5) and CRP, still negatively predicted the clinical stability of patients, with HRs (95% CI) of 0.33 (0.15–0.74, $P < 0.01$), 0.14 (95% CI 0.03–0.80, $P < 0.05$), and 0.99 (0.98–1.00, $P < 0.05$), respectively. However, the correlation between high CURB-65 scores (3–5) ($P = 0.497$), PCT ($P = 0.38$), and the patients' clinical stability was not significant (Table 3).

In 93 CAP patients, there are 21 patients who were measured the levels of sST2 at the moment of reaching clinical stability. Compare with at admission, the levels of sST2 of patients reaching clinical stability were significantly lower ($P < 0.05$). Moreover, all the levels of sST2 of patients reaching clinical stability were lower than the cutoff value of 72.8 ng/mL evaluated by the Youden index (Supplementary Figure 2), showing that serum sST2 concentration recover normal level if the patient reaches clinical stability.

Additive effect of sST2 on CURB-65 scores in the prediction of clinical stability

To explore whether sST2 can improve the power of CURB-65's risk stratification and prognostic evaluation, we compared the ability of two survival regression models in predicting the clinical stability of patients. Model 1 was a univariate Cox regression model with CURB-65 scores alone, whereas model 2 was a multivariate Cox regression model with CURB-65 scores and serum sST2 levels at admission. When comparing model 2 with model 1, the AUC were 0.96 and 0.89, respectively (all $P < 0.05$) (Figure 4(a)), with NRI (95% CI) of 1.18 (0.68–1.67, $P < 0.05$) and IDI (95% CI) of 0.16 (0.04–0.28, $P < 0.05$). Thus, model 2 can more accurately perform risk

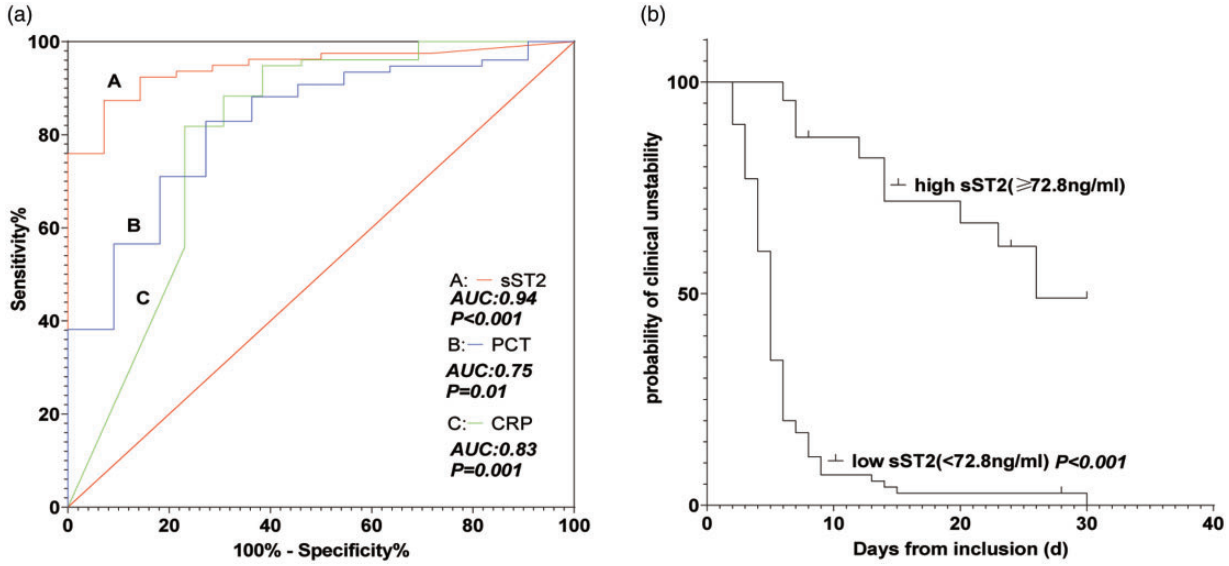

Figure 3. Performance of serum ST2 level in predicting the clinical stability of CAP patients at admission. A: (a) In the ROC curve analysis, the AUC (95% CI) of admission serum ST2 level that predicted the main outcome of CAP patients was 0.95 (0.90–0.99); (b) the AUC of PCT was 0.75 (0.54–0.95); (c) the AUC of CRP was 0.83 (0.70–0.96); B: Elevated sST2 level defined as ≥72.8 ng/mL; the Kaplan–Meier curve used to show the predicted clinical outcomes of CAP patients based on sST2 level, with HR (95% CI)of 0.13 (0.06–0.26), $P < 0.05$. (A color version of this figure is available in the online journal.)

Table 3. Effects of sST2 levels and CURB-65 on predicting clinical stability.

	Univariate analysis ($n = 93$)		Multivariate analysis ($n = 93$)	
	Hazard ratio (95% CI)	*P* value[a]	Hazard ratio (95% CI)	*P* value[a]
sST2 (>72.8 ng/mL)[b]	0.13 (0.06–0.26)	<0.05	0.33 (0.15–0.74)	<0.05
CURE-65 (3–5)	0.15 (0.07–0.33)	<0.05	0.71 (0.27–1.89)	0.497
PSI (5)	0.05 (0.01–0.19)	<0.05	0.14 (0.03–0.80)	<0.05
PCT	0.35 (0.18–0.69)	<0.05	0.82 (0.54–1.27)	0.38
CRP	0.98 (0.97–0.99)	<0.05	0.99 (0.98–1.00)	<0.05

95% CI, 95% confidence interval.
[a]*P* values were calculated using Cox regression analysis.
[b]The cut-off value for defining sST2 was optimized using the receiver operating characteristic curve analysis and calculating the Youden index.

classification in CAP patients when compared with model 1 (Figure 4(b) and (c)).

For an easy use of soluble ST2 as a prognostic predictor in a clinical setting, we proposed a combined index as CURB-65 + 1 (When serum sST2 levels ≥72.8 ng/mL at admission) or CURB-65 alone (When serum sST2 levels <72.8 ng/mL at admission). In model 1, patients with CURB-65 of 0–1 were defined as a low-risk group (n= 60), patients with CURB-65 of 2 as a moderate-risk group (n = 16), and patients with CURB-65 of 3–5 as a high-risk group (n = 17). Compared to the low-risk group, the HR (95% CI) of the moderate-risk and high-risk groups were 0.54 (0.30–0.98, P= 0.043) and 0.15 (0.07–0.33, $P < 0.05$), respectively. In model 2, patients with CURB-65 + 1 of 0–1 were defined as a low-risk group (n = 55), patients with CURB-65 of 2–3 as a moderate-risk group (n = 25), and patients with CURB-65 of 4–6 as a high- risk group (n = 13). Compared to the low-risk group, the HR (95% CI) of the moderate-risk and high-risk groups were 0.32 (0.19–0.55, $P < 0.05$) and 0.05 (0.01–0.15, $P < 0.05$), respectively.

We then evaluated the characteristics of patients in the different groups divided by model 2. The PSI scores in the high-risk and the moderate-risk groups were significantly higher than those in the low-risk group ($P < 0.05$). Serum sST2 levels in the high-risk group were significantly higher than those in the moderate-risk and low-risk groups ($P < 0.05$, $P = 0.03$, respectively), and those in the moderate-risk group were significantly higher than those in the low-risk group ($P < 0.05$). The laboratory indicators, including PCT, CRP, and SAA, in the high-risk group and the moderate-risk group were also significantly higher than those in the low-risk group (all $P < 0.05$). Furthermore, the probability of patients reaching clinical stability was 23.08% (3/13), 84% (21/25), and 100% (55/55) in high-risk group, moderate-risk group, and low-risk group, respectively.

Additive effect of sST2 on PSI scores in the prediction of clinical stability

To probe whether sST2 can facilitate the accuracy of PSI's risk stratification, we compared the power of two survival regression models in predicting patients' clinical stability. Model 3 is a univariate Cox regression with PSI scores alone, whereas model 4 was a multivariate Cox regression combined with PSI scores and serum sST2 levels at

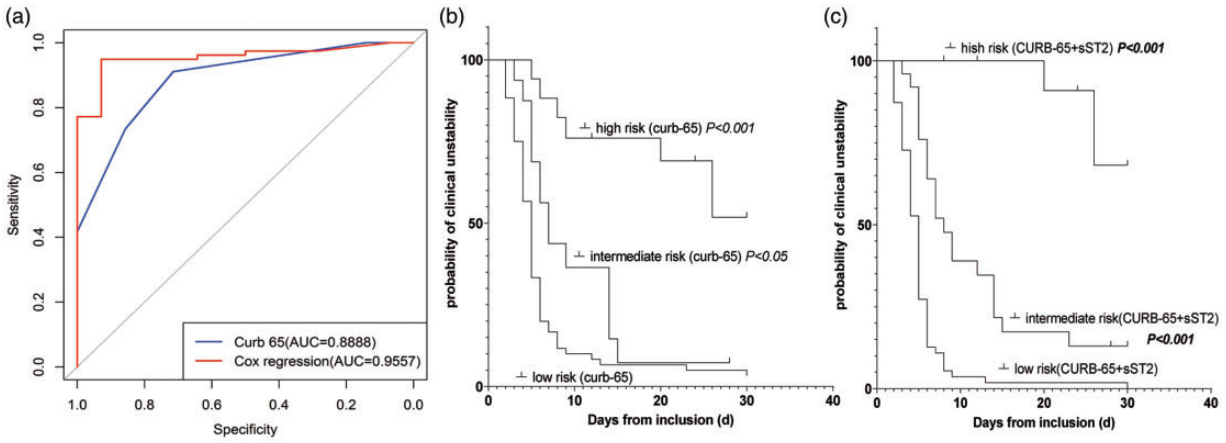

Figure 4. The established risk classification models using the CURB-65 score alone and CURB-65 score + sST2, respectively. (a) In the ROC curve analysis, the AUC of model 1 (CURB-65 score alone) and composite model (CURB-65 score + sST2) were 0.89 and 0.96, respectively, $P < 0.05$; (b) According to the CURB-65 score, the patients were divided into high-risk (3–5), medium-risk (2) and low-risk (0–1). Using the low-risk group as the control, the HR (95% CI, *P*) of the high-risk and medium risk groups were 0.15 (0.07–0.33, $P < 0.05$) and 0.54 (0.30–0.98, $P < 0.043$), respectively; (c) When sST2 $\geq$ 72.8 ng/mL, the composite index is CURB-65 + 1, or when sST2 $<$ 72.8 ng/mL, the index is CURB-65 alone. According to the CURB-65 + sST2 score, the patients were divided into high-risk (4–6), medium-risk (2–3) and low-risk (0–1). Using the low-risk group as the control, the HR (95% CI, *P*) of the high-risk and medium-risk groups were 0.05 (0.01–0.15, $P < 0.05$) and 0.32 (0.19–0.55, $P < 0.05$), respectively. (A color version of this figure is available in the online journal.)

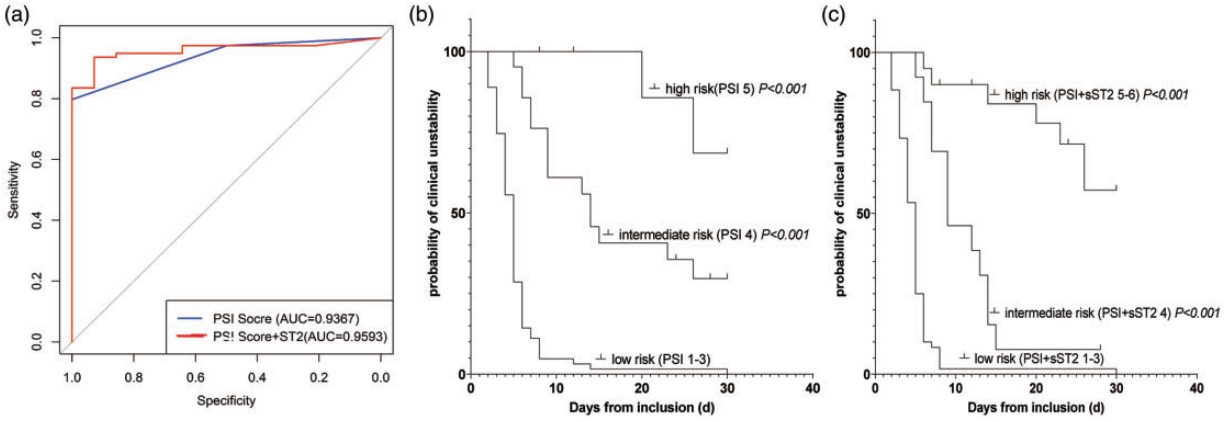

Figure 5. The established risk classification models using the PSI score alone and PSI score + sST2, respectively. (a) In the ROC curve analysis, the AUC of model 3 (PSI score alone) and composite model (PSI score + sST2) were 0.93 and 0.96, respectively, $P < 0.05$; (b) According to the PSI score, the patients were divided into high-risk (5), medium-risk (4) and low-risk (1–3). Using the low-risk group as the control, the HR (95% CI, *P*) of the high-risk and medium-risk groups were 0.05 (0.01–0.19, $P < 0.05$) and 0.18 (0.10–0.34, $P < 0.05$), respectively; (c) When sST2 $\geq$ 72.8 ng/mL, the composite index is PSI + 1, or when sST2 $<$ 72.8 ng/mL, the index is PSI alone. According to the PSI + sST2 score, the patients were divided into high-risk (5–6), medium-risk (4) and low-risk (1–3). Using the low-risk group as the control, the HR (95% CI, *P*) of the high-risk and medium-risk groups were 0.06 (0.03–0.14, $P < 0.05$) and 0.27 (0.14–0.53, $P < 0.05$), respectively. (A color version of this figure is available in the online journal.)

admission. When comparing model 4 with model 3, the AUC were 0.96 and 0.93, respectively (all $P < 0.05$) (Figure 5(a)), with NRI (95% CI) of 0.06 (0.0096–0.117, $P < 0.05$) and IDI (95% CI) of 0.06 (0.0093–0.1173, $P < 0.05$). Thus, compared with model 3, model 4 can more accurately perform the risk classification of CAP patients (Figure 5(b) and (c)).

To simplify the use of soluble ST2 as a prognostic predictor in a clinical setting, we defined a combined index as PSI + 1 (when serum sST2 levels $\geq$ 72.8 ng/mL at admission) or PSI alone (When serum sST2 levels $<$ 72.8 ng/mL at admission). In model 3, patients with PSI of 1–3 were defined as a low-risk group (n= 63), patients with PSI of 4 as a moderate-risk group (n= 21), and patients with PSI of 5 as a high-risk group (n= 9). Compared with the low-risk group, the HRs (95% CI) of the moderate-risk and high-risk groups were 0.18 (0.10–0.34, $P < 0.05$) and 0.05 (0.01–0.19, $P < 0.05$), respectively. In model 4, patients with PSI of 1–3 were defined as a low-risk group (n= 60), patients with PSI of 4 as a moderate-risk group (n = 13), and patients with PSI of 5–6 as a high-risk group (n= 20). Compared to the low-risk group, the HRs (95% CI) of moderate-risk and high-risk groups were 0.27 (0.14–0.53, $P < 0.05$) and 0.06 (0.03–0.14, $P < 0.05$), respectively.

We then evaluated patients' characteristics in the different groups divided by model 4. The PSI scores in the high-risk and the moderate-risk group were significantly higher than those in the low-risk group ($P < 0.05$). Serum sST2 levels in the high-risk group were significantly higher than those in the moderate-risk groups ($P < 0.05$), and the moderate-risk group were significantly higher than those in the low-risk group ($P < 0.05$). The laboratory indicators, including PCT, CRP, and SAA, in the high-risk group and

the moderate-risk group were significantly higher than those in the low-risk group (all $P < 0.05$). Furthermore, the probability of patients reaching clinical stability was 35% (7/20), 92.31% (12/13), and 100% (60/60) in the high-risk group, moderate-risk group, and low-risk group, respectively.

Discussion

Our study demonstrated that serum sST2 levels at admission could reflect disease severity in CAP patients and predict whether the patients reach clinical stability. We found that serum sST2 levels at admission of patients without clinical stability (137.95 vs. 14.00 ng/mL, $P < 0.05$) were significantly higher than those in patients with clinical stability. The serum sST2 levels at admission were positively correlated with not only PSI and CURB-65 scores, but also CAP, SAA, and CRP, which are authoritative indicators for infection severity. High serum sST2 levels ($\geq$ 72.8 ng/mL) could also negatively predict clinical stability. Moreover, we built models combined with sST2 levels at admission with CURB-65 and PSI, respectively, which abilities to classify risk and monitor the prognosis of CAP patients was significantly higher than those of PSI and CURB-65 scores alone.

Ours study showed that higher serum sST2 levels at-admission indicate a lower probability of the patient's restoring clinical stability. The ST2/IL-33 signaling pathway plays an important regulatory role in pulmonary infection. In mouse models of sepsis, IL-33 treatment can improve inflammation and reduce mortality.[26] Similarly, in a mouse model of COPD exacerbation caused by influenza virus infection, the administration of IL-33 increased the infiltration of neutrophils in the lungs, but the administration of sST2 decreased this infiltration.[27] Studies showed that the lung is the main cellular source of sST2 secretion,[28] and that there are many types of human lung cells (i.e. bronchocytes, alveolar epithelial cells, and vascular endothelial cells) that can release sST2 *in vitro*.[29] Moreover, pro-inflammatory cytokines can also promote the release of sST2. sST2 acts as a decoy receptor that can competitively bind to IL-33 and block the ST2/IL-33 signal axis. Therefore, we speculated that in lung infection diseases (Such as CAP), the combination of necrotic cell-released IL-33 and the cell membrane ST2L enhances the chemotaxis of neutrophils and other inflammatory cells to the lung, strengthening the inflammatory response of the lung and eliminating infectious pathogens. However, the pro-inflammatory cytokines and the damaged lung cells or tissues increased the secretion of sST2, blocked the ST2/IL-33 signal axis, and prevented the elimination of infectious pathogens through inflammation, leading to patients' poor prognosis. Therefore, sST2 can be used as a biomarker for CAP prognosis.

In this study, the sST2 levels of CAP patients at admission had good correlation with infection indices such as PCT and CRP. Recently, some well-designed studies have confirmed that PCT and CRP can be used to monitor the treatment responses of CAP patients. They could reduce the frequency and duration of antimicrobial agents' administration without increasing the mortality of patients and prevent the occurrence of antibiotic resistance in hospital environment.[30,31] Evelien et al. proved that PCT monitoring of CAP patients could reduce the 28-day mortality rate and one-year mortality rate by 5% and 7.4%, respectively.[30] The strong correlations between sST2, PCT, and CRP indicate that the time-course monitoring of sST2 in CAP patients also has the potential in guiding a safely shortening of the antibiotic treatment duration. However, more prospective studies are needed to confirm this hypothesis.

The CURB-65 and PSI scores are powerful tools to classify the risk and evaluate the prognosis of patients with pneumonia.[32,33] The CURB-65 score was derived and validated from the data of 1000 CAP inpatients and was found to be potentially effective in stratifying patients based on a 30-day mortality.[24] Currently, the CURB-65 score has been widely utilized in clinical practice and was recommended as a real-time decision support tool for CAP.[34] The PSI is a prognosis evaluation tool derived and validated from the data of 50,000 CAP patients by universally acknowledged methodological criteria. Through 20 clinical indices, the PSI divides patients into classes 1–5 to predict short-term mortality. Because of its accuracy, rigor, effectiveness and safety, the PSI has become a reference standard for CAP risk stratification.[33] In this study, sST2 combination with the CURB-65 score or the PSI at admission can establish more effective CAP risk classification models, and the results are in line with the study of Masato et al.[35] In the combined models, the prediction of high-risk indicates that the patients had a significant higher chance to have adverse hospital outcomes, including severe infection and adverse complications (acute respiratory distress syndrome, sepsis, septic shock, *et al.*), even if they were in a seemingly stable condition. The multivariate models, we believe, will be a potentially powerful tool for clinicians to manage adequate clinical interventions to CAP patients early.

However, there are some limitations in our study. Firstly, this is a small-scale and single-center study. Therefore, the evaluation sST2 role in the treatment and prognosis of CAP needs to be confirmed in a large-scale, multicenter cohort study. Secondly, the database only has the serum sST2 levels of CAP patients at admission so we are not able to study the dynamic changes of sST2 in CAP patients. Finally, this study is a retrospective study, and the lack of long-term follow-up information may compromise the reliability of our results.

The serum sST2 level at admission can predict the disease severity and the possibility of patient's restoring clinical stability of CAP patients, and thus can be used as an early monitoring index in CAP. The addition of sST2 level to the PSI score and the CURB-65 score could provide a more accurate risk classification and prognostic analysis for CAP patients.

AUTHORS' CONTRIBUTIONS

Conception and design of the research: BS and XDZ; Drafting the manuscript: YZe and MX; Samples collection and

detection: RL and NL; Acquisition of data: SS, TZ, and PZ; Statistical analysis: YZe, TZ and YZh; Analysis and interpretation of data: HHa, SS and PZ.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

This study was approved by the ethic committee of The First Affiliated Hospital of Guangzhou Medical University (Reference number: GYFYY-2016–73).

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by the National Natural Science Foundation of China (Project No.: 81871736), Guangdong Science and Technology Fund (Project No.: 2020B1111300001), Guangzhou Institute of Respiratory Health Open Project (Funds provided by China Evergrande Group) (Project No.:2020GIRHHMS04), Science and Technology Project of Guangzhou (Project No.: 20181A011061). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. No additional external funding was received for this study.

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Supplemental material

Supplementary material for this article is available online.

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(Received March 11, 2021, Accepted June 1, 2021)