Soluble ST2 in serum predicts the prognosis of idiopathic pulmonary fibrosis: a retrospective study

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Background: Idiopathic pulmonary fibrosis (IPF) is a heterogeneous and progressive fibrosing interstitial lung disease with a poor prognosis. However, there are currently no effective biomarker that can reliably predict the prognosis for IPF in clinic. The serum level of soluble suppression of tumorigenicity-2 (sST2), which is involved in the immune response, has proven to be a prognostic predictor for various diseases. Previous studies have confirmed that the immune dysfunction plays an important role in the pathogenesis of IPF and the serum sST2 concentrations in patients with IPF are elevated. However, the relationship between sST2 and the prognosis of IPF remains unknown.

Methods: A total of 83 patients with IPF and 20 healthy controls from 2016 to 2021 were enrolled and demographic variables, indices of lung function testing as well as the biomarkers including the sST2 were obtained at baseline. During follow-up, the primary endpoint was defined as all-cause death and clinical deterioration. Cox hazard models and Kaplan-Meier method were used to assess the prognostic value of various indices including sST2.

Results: Mean duration of follow-up was 29 months, during which 49 patients had an event, and of them, 35 patients died. The sST2 level was higher in the IPF patients compared with the healthy controls. Although the sST2 level did not directly predict all-cause death in the present study, it was proved to be an independent predictor of event-free survival. Multivariate forward stepwise model which was adjusted by age, sex, and body surface area (BSA) showed that the overexpression of sST2 increased the hazard ratio [1.005, 95% confidence interval (CI): 1.001–1.010]. A higher sST2 serum level heralded more deterioration and the poor outcomes. Moreover, the effect of sST2 on the prognosis of IPF may not necessarily involve the development of IPF-related pulmonary hypertension (PH).

Conclusions: In our study, the sST2 serum level was significantly elevated and a higher serum level of sST2 predicted more deterioration and poor outcomes in patients with IPF. Thus, sST2 can serve as a valuable prognostic biomarker for the outcome of IPF. However, further multicenter clinical trials of larger sample size are needed in the future.

Keywords: Idiopathic pulmonary fibrosis (IPF); prognostic factors; soluble suppression of tumorigenicity-2 (sST2)

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Introduction

Idiopathic pulmonary fibrosis (IPF), a type of interstitial lung disease (ILD), is characterized by irreversible fibrosis caused by inflammation, fibroblast accumulation, and excessive collagen deposition, resulting in gradual deterioration and poor prognosis, and few effective therapeutics exist (1). The progression of the disease and the treatment responses vary for IPF patients, increasing the uncertainty of prognosis. Therefore, indices acquired during the first diagnosis especially that can assist with prognosis are urgently needed. Hence, predictors for predicting the prognosis of IPF and monitoring its progress are keenly awaited. Indices of lung function have proved to have prognostic significance in IPF (2). However, the universality and feasibility of the tests prevent them from being widely used in prognosis prediction. Therefore, the demand for useful biomarkers with an early diagnostic potential has increased manifold. Although several exciting candidates have been reported as biomarkers for IPF, none of them have been widely used in IPF (3). Therefore, indices that have been widely used in clinical practice and can assist with prognosis of the IPF are urgently needed.

Suppression of tumorigenicity-2 (ST2) is an interleukin-1 (IL-1) receptor family member that exists in both transmembrane (ST2L) and soluble (sST2) isoforms. sST2 in serum has been identified as a valuable prognostic biomarker in various diseases and has been widely used especially in the clinical practice of the cardiovascular diseases (4-6). ST2 is associated with the immune response (7), and in ILD alveolar epithelial injury is the initial injury process that induces the immune response and the secondary fibrosis (8). Various cytokines, including IL-33, are involved in the innate immune response of the epithelium that eventually leads to the development of IPF (8). ST2 cross-talk with IL-33 has long been known to play a pivotal role in lung fibrosis diseases, affecting the balance between extensive inflammation and tissue regeneration which leads to the remodeling that is the hallmark of fibrosis (9,10). Moreover, sST2 levels increase in the serum during acute exacerbation of IPF, which may reflect disease severity (11). All the previous studies indicated that sST2 may be closely related to the progression of IPF, but, the exact relationship between sST2 and the prognosis of IPF is yet to be clearly elucidated.

Therefore, the primary aim of the present study was to investigate whether the sST2 level in serum is associated with the clinical outcomes of IPF patients and its prognostic value. We present the following article in accordance with the STARD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3215/rc).

Methods

Study subjects

The Department of Respiratory and Critical Care Medicine, Nanjing Drum Tower Hospital, Affiliated Drum Tower Clinical Medical College of Nanjing Medical University retrospectively enrolled 83 patients with IPF (mean age 60.47±10.39 years, 65.3% male) and 20 healthy (mean age 59.55±13.82 years, 65.0% male) controls from between February 2016 to September 2021. Diagnostic preliminaries included clinical history, physical examination, blood tests, lung function tests, high-resolution computed tomography (HRCT) scan of the thorax, and echocardiography. Diagnosis of IPF was made according to the recent American Thoracic Society/European Respiratory Society consensus statement (12), which is detection of the usual pattern of interstitial pneumonia on HRCT, excluding patients with other known causes of interstitial lung disease, such as domestic or occupational environmental exposure, connective tissue disease with autoimmune features, and drug toxicity. The criterion for pulmonary hypertension (PH) in the present study was pulmonary artery systolic pressure ≥35 mmHg without either abnormal structure of the right heart or right heart failure as assessed by echocardiography. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of the Nanjing Drum Tower Hospital (No. 2021-390-01). Informed consent was taken from all the patients.

Assessment of patients

Demographic variables, such as sex, age, body surface area (BSA), and indices of lung function testing, were obtained at baseline. Venous blood samples for analysis including the lymphatic cellular subgroup were taken only for study purposes. Clinical decision making was made independent of biomarkers. The levels of sST2 in serum were measured in banked serum samples via a highly sensitive sandwich monoclonal immunoassay (Presage™ ST2 assay, Critical Diagnostics, San Diego, CA). The blood used in the present study had been subjected to a single freeze–thaw cycle. All indices were measured by personnel blinded to the patients' clinical data.

Outcomes

The primary endpoint was defined as all-cause death and clinical deterioration, including death and rehospitalization due to rapidly worsening IPF and acute exacerbations (AE)-IPF. AE-IPF was diagnosed according to the criteria described by Collard *et al.* in 2016 (13). Briefly: (I) previous or concurrent diagnosis of IPF; (II) acute worsening or development of dyspnea typically of <1 month in duration; (III) HRCT findings of new bilateral ground-glass opacity and/or consolidation superimposed on a background pattern consistent with usual interstitial pneumonia; and (IV) deterioration not fully explained by cardiac failure or fluid overload. Follow-up of each patient was conducted at 6-monthly intervals. The status of the patients was checked by the medical records in the outpatient clinic or by phone call. Survival and event-free survival were repeatedly estimated during every follow-up from the date of diagnosis to February 2021. The survival was calculated as the number of months from IPF diagnosis to death and the event-free survival was calculated as the number of months from IPF diagnosis to clinical deterioration. Patients lost to follow-up were censored as alive on the last day of contact.

Statistical analysis

All results are expressed as mean ± SD or median (interquartile range) for continuous variables and as the absolute number for categorical variables. Kolmogorov-Smirnov test was used to test the normality of the continuous variables. Comparisons were performed using the independent-sample *t*-test, paired *t*-test, or Mann-Whitney U test for continuous variables and chi-square test for categorical variables. Univariate and multivariate Cox proportional hazards models were performed to evaluate the prognostic impact on survival of all variables of interest. Age, sex, and BSA were adjusted to ascertain the independent prognostic role of indices involved in the study. The Receiver-operating characteristic (ROC) curves were used to select the cut-off values for independent predictors with maximum sensitivity and specificity. Kaplan-Meier method and log-rank test were used to perform the survival analyses. All tests were two-sided and performed at a significance level of 0.05. The main analysis was performed using SPSS (Statistic Package for Social Science, Chicago, IL, USA) version 19.0.

Results

Comparison of characteristics and indices between the IPF patients and healthy controls

A total of 83 IPF patients and 20 healthy people were included in the study, with no there were no apparent differences for age, BSA, smoking, sex or lactate dehydrogenase (LDH) (*Table 1*). However, the CD4 T-cell counts were lower in the IPF patients, and C-reactive protein (CRP) levels higher in the IPF patients compared with the healthy group. Above all, sST2 was higher in the IPF patients with significant statistical difference compared with the healthy controls. Among the IPF patients, the mean follow-up was 29 months, during which 49 (59.03%) patients had an event: 36 patients required rehospitalization due to rapid worsening IPF or AE-IPF, and 13 patients required additional medication due to clinical worsening. Of the 49 patients with an event, 35 died. No patient was lost to follow-up, giving a follow-up rate of 100%.

Comparison of indices between the survival and non-survival groups

Table 2 presents the comparison of indices between the survival and non-survival groups of IPF patients. There were no differences for age, BSA, sex, smoker, N-terminal pro-B-type natriuretic peptide (NT-pro-BNP), predicted forced expiratory volume in 1 second, LDH, CRP, CD4 T cells, CD8 T cells, B cells, arterial oxygen saturation (SaO_2) or sST2 between the non-survival and survival groups. However, forced vital capacity (FVC)/predicted, diffusing capacity of the lung for carbon monoxide (DLCO)/predicted and the natural killer (NK) cell count were higher

Table 1 Comparison of the characteristics of the IPF patients and healthy controls

Characteristics	Controls (n=20)	IPF (n=83)	P value
Age, years	59.55±13.82	60.47±10.39	0.783
BSA	1.61±0.15	1.69±0.16	0.065
Male	13 (65.00)	54 (65.06)	0.399
Smoker	10 (50.00)	40 (48.19)	0.961
LDH, U/L	145 (177, 241)	146 (203, 307)	0.177
CRP, mg/L	5.00 (6.00, 7.00)	17.00 (23.00, 42.00)	<0.001
CD4 T cell, 10^9/L	0.49 (0.25, 0.80)	0.28 (0.14, 0.47)	0.036
CD8 T cell, 10^9/L	0.28 (0.20, 0.40)	0.23 (0.16, 0.41)	0.640
NK cell, 10^9/L	0.30 (0.10, 0.47)	0.22 (0.13, 0.40)	0.726
B cell, 10^9/L	0.19 (0.11, 0.27)	0.20 (0.11, 0.28)	0.726
sST2, ng/mL	22.25 (11.27, 56.00)	67.50 (12.38, 124.50)	0.026

The data are shown as n (%), mean ± SD, and median (interquartile range). IPF, idiopathic pulmonary fibrosis; BSA, body surface area; LDH, lactate dehydrogenase; CRP, C-reactive protein; NK, natural killer; sST2, soluble suppression of tumorigenicity-2.

in the survival group of IPF patients with a significant statistical difference compared with the non-survival group. Moreover, event-free time was shorter for the non-survival group with statistical significance. Medication including N-acetyl-L-cysteine (NAC), pirfenidone, and nintedanib as well as combination therapy was used by the IPF patients in the present study. There were no apparent differences in medication use between the non-survival and survival groups (*Table 2*).

Factors influencing survival in IPF patients

In the univariate Cox proportional hazards analysis, event-free survival time, FVC/predicted, DLCO/predicted as well as the NK cell count were related to survival (P<0.1). However, age sex and BSA were not predictors of survival. In the multivariate forward stepwise, the model was adjusted by age, sex, and BSA, and of all these indices, event-free survival time, FVC/predicted and DLCO/predicted were proved to be independent predictors of survival (*Table 3*).

Receiver-operating characteristic curves for all-cause death

ROC curves were plotted for event-free survival time, FVC/predicted and DLCO/predicted and the results are shown in *Table 4*. For predicting all-cause death, event-free survival time <15.18 months had a sensitivity of 73.9% and specificity of 71.4%, FVC/predicted <67.45% had a sensitivity of 70.8% and specificity of 68.6% and the cut-off value for DLCO/predicted was 66.50% with a sensitivity of 52.1% and specificity of 77.1%.

Comparison of indices between the event-free and event groups

The differences in the indices between the event-free and event groups are presented in *Table 5*. Among the indices, FVC/predicted, DLCO/predicted and SaO_2 were higher in the event-free group of IPF patients with significant statistical differences compared with the event group. Moreover, the CD4 T cell and NK cell counts, as well as the SaO_2, were lower in the event group with statistical significance. Above all, the sST2 serum levels were significantly higher in the event group compared with the event-free group. No differences between groups were found for the other indices.

Factors influencing event-free survival in IPF patients

We further investigated the indices related to event-free survival. In the univariate Cox proportional hazards analysis, FVC/predicted, DLCO/predicted, and the CD4 T and NK cell counts as well as the sST2 were related to event-free survival (P<0.1). The multivariate forward

Table 2 Comparison of the characteristics of the survival and non-survival subgroups in idiopathic pulmonary fibrosis

Parameters	Survival (n=48)	Non-survival (n=35)	P value
Age, years	60.69±12.00	60.17±7.83	0.820
BSA	1.67±0.16	1.73±0.16	0.092
Male	28 (58.33)	26 (74.28)	0.132
Smoker	20 (41.66)	20 (57.14)	0.163
NT-pro-BNP, pg/mL	89.5 (72.50, 137.00)	98.00 (58.75, 120.50)	0.898
Event-free survival, m	26.25 (12.30, 41.44)	8.38 (3.02, 21.71)	<0.001
FVC/predicted, %	76.00 (67.43, 84.75)	66.00 (57.30, 74.00)	0.001
FEV1/predicted, %	87.00 (78.00, 87.75)	87.00 (78.00, 91.00)	0.138
DLCO/predicted, %	67.00 (55.25, 76.00)	56.00 (51.00, 66.00)	0.005
LDH, U/L	204.00 (145.00, 266.00)	194.0 (147.00, 405.00)	0.586
CRP, mg/L	22.00 (17.00, 34.00)	32.00 (18.00, 44.00)	0.252
CD4 T cells, 10^9/L	0.29 (0.15, 0.59)	0.24 (0.11, 0.44)	0.272
CD8 T cells, 10^9/L	0.16 (0.23, 0.39)	0.23 (0.17, 0.49)	0.726
NK cells, 10^9/L	0.30 (0.16, 0.47)	0.18 (0.11, 0.27)	0.028
B cells, 10^9/L	0.22 (0.11, 0.33)	0.18 (0.11, 0.26)	0.266
SaO_2, %	88.60 (85.90, 91.80)	88.90 (87.00, 93.48)	0.455
sST2, ng/mL	33.30 (12.37, 100.06)	88.10 (13.30, 151.10)	0.114
Medications			0.790
NAC	27 (56.25)	17 (48.57)	
Pirfenidone	5 (10.41)	6 (17.14)	
Nintedanib	4 (8.33)	4 (11.43)	
NAC + pirfenidone	8 (16.67)	4 (11.43)	
NAC + nintedanib	4 (8.33)	4 (11.43)	

The data are shown as n (%), mean ± SD, and median (interquartile range). BSA, body surface area; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; DLCO, diffusing capacity of the lung for carbon monoxide; LDH, lactate dehydrogenase; CRP, C-reactive protein; NK, natural killer; SaO_2, arterial oxygen saturation; sST2, soluble suppression of tumorigenicity-2; NAC, N-acetyl-L-cysteine.

stepwise model was also adjusted by age, sex, and BSA. Among these indices, FVC/predicted as well as the sST2 were independent predictors of event-free survival (*Table 6*).

Receiver-operating characteristic curves for event

ROC analysis was conducted for evaluating the sensitivity and specificity of sST2 and FVC/predicted as predictors of event-free survival (*Table 7*). The ROC-optimal sST2 cut-off value was 56.40 ng/mL with a sensitivity and specificity of 71.4% and 73.5% respectively. In addition, FVC/predicted could also be a predictor of events, with the initial cut-off value of 76.95% for predicting death (sensitivity and specificity of 73.7% and 47.1% respectively).

Kaplan-Meier survival and event-free survival analyses

Kaplan-Meier survival curves were plotted according to the cut-off values of event-free survival time, FVC/predicted, and NK cell count by ROC analysis. Patients with event-free survival time ≥15.18 months had a significantly better prognosis than those with event-free survival time

Table 3 Univariate and multivariate analyses results relating survival to selected indices

Parameters	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age	0.997 (0.963–1.031)	0.852	–	–
Sex	0.694 (0.325–1.485)	0.347	–	–
BSA	4.519 (0.643–31.762)	0.129	–	–
Smoker	0.702 (0.359–1.372)	0.301	–	–
NT-pro-BNP	1.001 (1.000–1.003)	0.169	–	–
Event-free survival	0.943 (0.918–0.969)	<0.001	0.939 (0.910–0.969)	<0.001
FVC/predicted	0.949 (0.925–0.973)	<0.001	0.958 (0.933–0.984)	0.002
FEV1/predicted	1.028 (0.987–1.071)	0.182	–	–
DLCO/predicted	0.966 (0.939–0.993)	0.015	0.968 (0.944–0.993)	0.013
LDH, U/L	1.002 (0.999–0.004)	0.227	–	–
CRP, mg/L	1.006 (0.998–1.015)	0.122	–	–
CD4 T cells, 10^9/L	0.450 (0.154–1.318)	0.111	–	–
CD8 T cells, 10^9/L	1.597 (0.346–7.376)	0.548	–	–
NK cells, 10^9/L	0.166 (0.022–1.265)	0.083	–	–
B cells, 10^9/L	0.253 (0.031–2.092)	0.202	–	–
SaO_2, %	0.976 (0.908–1.046)	0.506	–	–
sST2, ng/mL	1.004 (0.999–1.009)	0.137	–	–

BSA, body surface area; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; DLCO, diffusing capacity of the lung for carbon monoxide; LDH, lactate dehydrogenase; CRP, C-reactive protein; NK, natural killer; SaO_2, arterial oxygen saturation; sST2, soluble suppression of tumorigenicity-2; HR, hazard ratio; CI, confidence interval.

Table 4 AUC and cut-off values for the independent predictors in patients with idiopathic pulmonary fibrosis

Variables	Cut-off value	Sensitivity	Specificity	AUC	95% CI	P value
Event-free survival (months)	15.18	0.739	0.714	0.748	0.643–0854	<0.001
FVC/predicted (%)	67.45	0.708	0.686	0.715	0.204–0.443	0.006
DLCO/predicted (%)	66.50	0.521	0.771	0.680	0.563–0.797	0.005

FVC, forced vital capacity; DLCO, diffusing capacity of the lung for carbon monoxide; AUC, area under receiver-operating characteristic curves; CI, confidence interval.

<15.18 months (*Figure 1*). *Figure 2* shows that patients with FVC/predicted ≥67.45% had significantly better survival, and the significant survival advantage in the patients with DLCO/predicted ≥66.50% is shown in *Figure 3*.

Similar results were also deduced from the cut-off values of FVC/predicted and sST2, which were related to event-free survival. A significant event-free survival advantage existed for patients with FVC/predicted ≥76.95% as well as sST2 <56.40 ng/mL (*Figures 4*,*5*). The combination of these two independent predictors identified subgroups with a significantly different probability of events. *Figure 6* shows that the subgroup with FVC/predicted ≥76.95% and sST2 <56.40 ng/mL had significantly better event-free survival than all the other three subgroups. No statistically significant difference was found in the comparison of other subgroups.

Table 5 Comparison of the characteristics between the event-free and event subgroups of patients with idiopathic pulmonary fibrosis

Parameters	Event-free (n=34)	Event (n=49)	P value
Age, years	59.29±12.10	61.28±9.06	0.839
BSA	1.67±0.16	1.70±0.16	0.360
Male	20 (58.82)	34 (69.39)	0.321
Smoker	13 (38.23)	27 (55.10)	0.130
NT-pro-BNP, pg/mL	89.50 (70.75, 138.25)	94.00 (60.25, 119.50)	0.946
FVC/predicted, %	75.00 (66.00, 86.00)	67.40 (58.65, 76.20)	0.039
FEV1/predicted, %	82.00 (78.00, 87.00)	87.00 (78.00, 89.00)	0.056
DLCO/predicted, %	67.00 (61.25, 76.00)	60.00 (53.00, 67.00)	0.009
LDH, U/L	204.00 (145.00, 309.00)	197.00 (146.00, 325.00)	0.963
CRP, mg/L	22.00 (17.00, 34.00)	32.00 (17.00,43.00)	0.436
CD4 T cells, 10^9/L	0.36 (0.19, 0.80)	0.27 (0.12, 0.43)	0.049
CD8 T cells, 10^9/L	0.22 (0.16, 0.35)	0.24 (0.12, 0.48)	0.620
NK cells, 10^9/L	0.34 (0.15, 0.56)	0.20 (0.12, 0.29)	0.019
B cells, 10^9/L	0.21 (0.11, 0.36)	0.19 (0.11, 0.27)	0.423
SaO_2, %	90.25 (87.40, 93.65)	88.00 (85.70, 91.45)	0.041
sST2, ng/mL	17.77 (11.18, 78.16)	89.40 (31.80, 149.20)	0.002
Medications			0.623
NAC	21 (61.76)	23 (46.94)	
Pirfenidone	3 (8.82)	8 (16.33)	
Nintedanib	2 (5.88)	6 (12.24)	
NAC + pirfenidone	5 (14.70)	7 (14.29)	
NAC + nintedanib	3 (8.82)	5 (10.20)	

The data are shown as n (%), mean ± SD, and median (interquartile range). BSA, body surface area; NT-pro-BNP: N-terminal pro-B-type natriuretic peptide; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; DLCO, diffusing capacity of the lung for carbon monoxide; LDH, lactate dehydrogenase; CRP, C-reactive protein; NK, natural killer; SaO_2, arterial oxygen saturation; sST2, soluble suppression of tumorigenicity-2; NAC, N-acetyl-L-cysteine.

Relationship between sST2 and PH in IPF patients

We further explored the relationship between sST2 and PH. As shown in *Figure 7*, no obvious linear relationship was found between sST2 and NT-pro-BNP, which is a primary prognostic index for PH (P≥0.05). Further comparison was made between the PH and no-PH groups of IPF patients. As shown in *Table 8*, the NT-pro-BNP levels were significantly higher in the PH group, but no statistical difference was found for the other indices, including sST2, as we expected.

Discussion

We found some significant results, and to the best of our knowledge, this is the first study to reveal the exact relationship between sST2 and the prognosis of IPF patients. Generally, sST2 was significantly higher in the IPF patients compared with healthy controls. Nevertheless, no statistically significant difference was found in sST2 between the survival and non-survival groups of IPF patients. Event-free survival time, as well as the FVC/predicted and DLCO/predicted, were found to be independent predictors

Table 6 Univariate and multivariate analyses results relating event-free survival to selected indices

Parameters	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age	0.997 (0.963–1.031)	0.852	–	–
Sex	0.694 (0.325–1.485)	0.347	–	–
BSA	4.519 (0.643–31.762)	0.129	–	–
Smoker	0.702 (0.359–1.372)	0.301	–	–
NT-pro-BNP	1.000 (0.999–1.002)	0.656	–	–
FVC/predicted	0.972 (0.950–0.993)	0.011	0.976 (0.955–0.998)	0.036
FEV1/predicted	1.028 (0.993–1.065)	0.116	–	–
DLCO/predicted	0.977 (0.954–1.001)	0.059	–	–
LDH	1.001 (0.999–1.004)	0.401	–	–
CRP	1.002 (0.994–1.010)	0.659	–	–
CD4 T cells	0.378 (0.156–0.915)	0.031	–	–
CD8 T cells	2.558 (0.638–10.253)	0.185	–	–
NK cells	0.171 (0.035–0.835)	0.029	–	–
B cells	0.451 (0.077–2.653)	0.378	–	–
SaO_2	0.949 (0.895–1.1007)	0.085	–	–
sST2	1.006 (1.002–1.010)	0.005	1.005 (1.001–1.010)	0.015

BSA, body surface area; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; DLCO, diffusing capacity of the lung for carbon monoxide; LDH, lactate dehydrogenase; CRP, C-reactive protein; NK, natural killer; SaO_2, arterial oxygen saturation; sST2, soluble suppression of tumorigenicity-2; HR, hazard ratio; CI, confidence interval.

Table 7 AUC and cut-off values for the independent predictors in patients with idiopathic pulmonary fibrosis

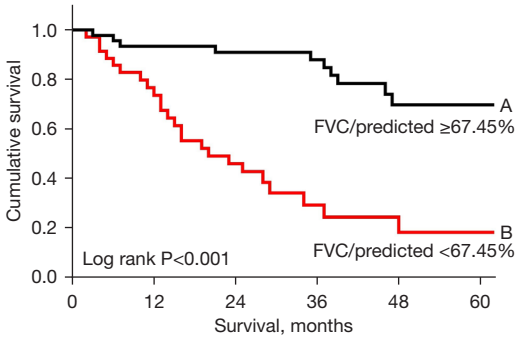
Variables	Cut-off value	Sensitivity	Specificity	AUC	95% CI	P value
sST2	56.4	0.714	0.735	0.697	0.580–0814	0.002
FVC/predicted	76.95	0.737	0.471	0.645	0.522–0.769	0.025

AUC, area under receiver-operating characteristic curves; sST2, soluble suppression of tumorigenicity-2; FVC, forced vital capacity.

of survival. Further investigation proved that sST2 and FVC/predicted were independent predictors of event-free survival, which indicated that sST2 affects the prognosis of IPF patients. In addition, the current study proved that the effect of sST2 on the prognosis of IPF may not be related to the development of PH.

IPF is the most common form of ILD with a rising incidence (14). The mortality rate in IPF is high with a reported median survival of 2–3 years from diagnosis and shows no improvement based on historical data and more recent evidence (15-18). In line with the previous registries, the present study showed that the median survival time was 29 months. Antifibrotic therapies have become increasingly available and improved the prognosis of IPF patients (19), but in the present study no differences were observed in regard to medications between the survival and non-survival groups or between the event and event-free groups possibly because only a few people enrolled in the study used the effective antifibrotic therapies.

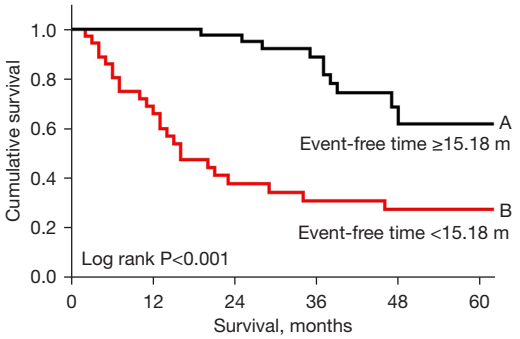
Because IPF is a heterogeneous disease with a variable course, predicting disease outcomes is difficult. Indices of lung function at both baseline and functional decline in the



No. at risk

Subgroup A	47	44	36	29	15
Subgroup B	36	25	15	7	4

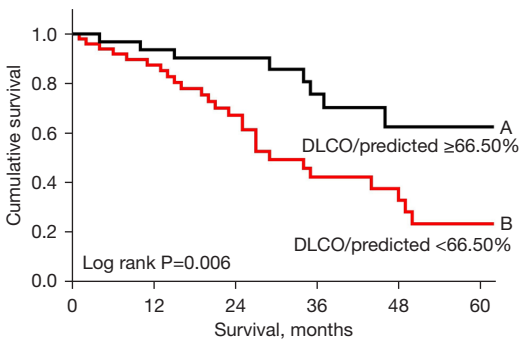
Figure 1 Cumulative survival (Kaplan-Meier) according to the cut-off value of FVC/predicted in idiopathic pulmonary fibrosis patients. FVC/predicted ≥67.45% had significantly better survival. FVC, forced vital capacity.



No. at risk

Subgroup A	45	45	39	26	10
Subgroup B	38	23	12	10	9

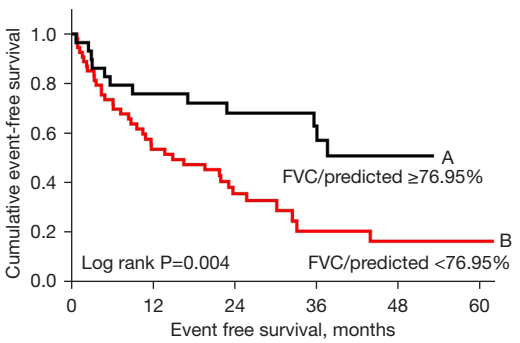
Figure 3 Cumulative survival (Kaplan-Meier) according to the cut-off value of event-free time in idiopathic pulmonary fibrosis patients. Event-free time ≥15.18 months had significantly better survival.



No. at risk

Subgroup A	33	30	25	15	8
Subgroup B	50	39	26	21	11

Figure 2 Cumulative survival (Kaplan-Meier) according to the cut-off value of DLCO/predicted in idiopathic pulmonary fibrosis patients. DLCO/predicted ≥66.50% had significantly better survival. DLCO, diffusing capacity of the lung for carbon monoxide.



No. at risk

Subgroup A	29	23	18	12	2
Subgroup B	54	27	15	4	2

Figure 4 Cumulative event-free survival (Kaplan-Meier) according to the cut-off value of FVC/predicted in idiopathic pulmonary fibrosis patients. FVC/predicted ≥76.95% had significantly better event-free survival. FVC, forced vital capacity.

progression of the disease have proved to have prognostic significance in IPF; that is, lower FVC and DLCO at baseline herald greater decline in lung function and poor prognosis (20,21). In accordance with that, our present study found that the baseline FVC and DLCO were lower in the non-survival group. Moreover, both indices were identified as independent predictors of all-cause death. However, regarding event-free survival, among the indices of lung function only FVC anticipated exacerbation events in the IPF patients. On the other hand, a prognostic study of IPF argued that baseline lung function alone is a poor predictor of mortality (19). The universality of lung function tests and the precision of the results being highly reliant on the cooperation of patients also prevent these indices from being used as prognostic predictors in clinical practice.

Because of the shortcomings of the lung function tests, biomarkers that predict disease endpoints, including disease presence, prognosis, and/or treatment response, are receiving increasing attention for IPF. The immune system plays a vital role in the progression of the disease; it is uncontrolled immune responses and imbalance of the

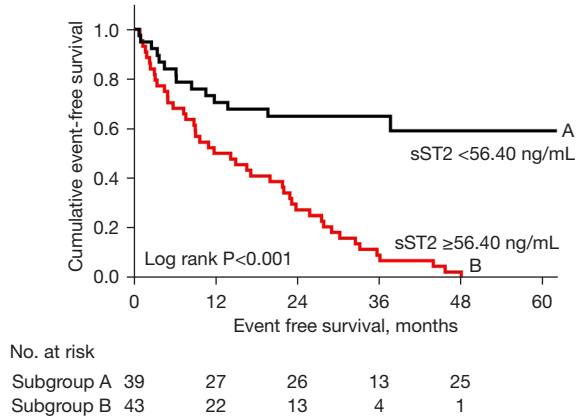


Figure 5 Cumulative event-free survival (Kaplan-Meier) according to the cut-off value of sST2 in idiopathic pulmonary fibrosis patients. sST2 <56.40 ng/mL had significantly better event-free survival. sST2, soluble suppression of tumorigenicity-2.

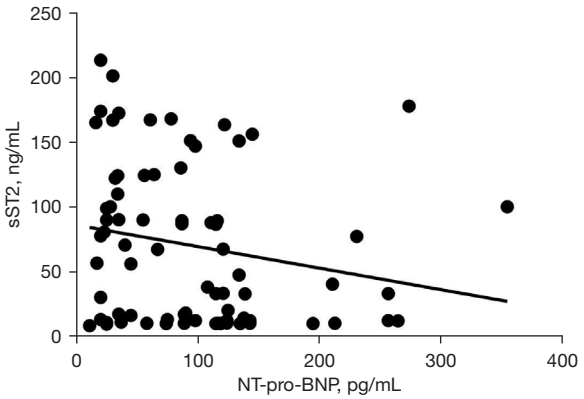


Figure 7 No linear relationship between NT-pro-BNP and sST2 in idiopathic pulmonary fibrosis patients (P>0.05). NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; sST2, soluble suppression of tumorigenicity-2.

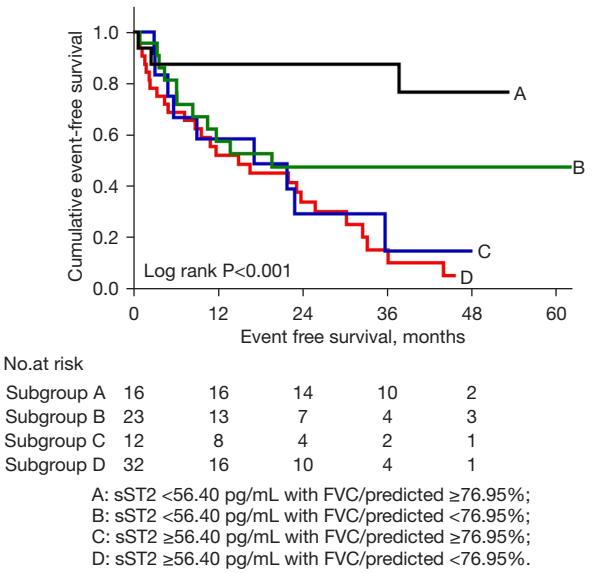


Figure 6 Cumulative event-free survival (Kaplan-Meier) according to the combined cut-off values of FVC/predicted and sST2 in idiopathic pulmonary fibrosis patients. sST2 <56.40pg/mL with FVC/predicted ≥76.95% had significantly better survival than in the other subgroups. FVC, forced vital capacity; sST2, soluble suppression of tumorigenicity-2.

injury-inflammation-repair process that drives the initiation and progression of IPF, while the regulatory immune system controls the pathogenic immune responses, regulates inflammation, and modulates the transition of inflammation to fibrosis (22). Hou *et al.* reported that activation of the regulatory T-cell proportion inhibited the proliferation of CD4 T-cells *in vitro* and correlated with the severity of IPF (23). In line with that study, we found that the CD4 T-cell proportion was lower in the IPF patients compared with the healthy controls, and the CD4 T-cell number in the events group of IPF patients was also lower than in the non-event group. However, CD4 T-cell count showed no prognostic significance in either survival or event-free survival. A past prospective cohort study of IPF showed that NK cell depletion and dysfunction in the peripheral circulation are closely associated with the severity of fibrosis (24). Our study proved that the IPF patients had lower NK cell counts than the healthy controls. Above all, both the non-survival group and the event group showed lower NK cell counts, which confirmed the close relation between NK cells and the severity of the disease. However, none of these immune biomarkers, which frequently used in clinical practice, were found to have prognostic significance in our study.

ST2, including both ST2L and sST2, is expressed in several cells under different conditions and has various triggers involved in the pathogenesis of various diseases including cancer, and inflammatory and cardiac diseases (7). The immune response leads to secondary fibrosis, which plays a pivotal role in the progression of fibrotic diseases (8). Cytokines, including IL-33, which is the ligand of ST2, are involved in this process. A recent study revealed that upregulated IL-33 is closely related to several chronic lung fibrotic diseases, but especially IPF (25-28). By signaling through ST2, IL-33 recruits and directs inflammatory cell

Table 8 Comparison between the PH and no-PH subgroups in idiopathic pulmonary fibrosis

Parameters	PH (n=25)	No-PH (n=58)	P value
Age, years	58.44±11.40	61.35±9.90	0.275
BSA	1.64±0.15	1.70±0.16	0.260
Male	13 (52.00)	41 (70.68)	0.101
Smoker	12 (48.00)	28 (48.28)	0.982
NT-pro-BNP, pg/mL	139.00 (48.50, 308.00)	89.00 (67.00, 102.00)	0.005
Survival, m	19.00 (7.50, 46.00)	34.50 (19.75, 47.00)	0.407
Event-free survival, m	14.13(5.24, 28.67)	21.82 (4.89, 34.87)	0.410
FVC/predicted, %	69.00 (63.50, 77.25)	73.00 (59.20, 82.00)	0.667
FEV1/predicted, %	87.00 (78.00, 89.00)	87.00 (78.00, 89.00)	0.662
DLCO/predicted, %	66.00 (55.50, 75.00)	65.00 (54.00, 69.00)	0.338
LDH, U/L	197.00 (175.50, 259.00)	203.50 (145.00, 345.00)	0.980
CRP mg/L	23.00 (14.00, 34.00)	23.00 (18.00, 43.00)	0.253
CD4 T cells, 10^9/L	0.20 (0.12, 0.44)	0.31 (0.17, 0.50)	0.165
CD8 T cells, 10^9/L	0.26 (0.19, 0.49)	0.21 (0.12, 0.39)	0.147
NK cells, 10^9/L	0.17 (0.09, 0.40)	0.22 (0.15, 0.42)	0.232
B cells, 10^9/L	0.17 (0.11, 0.35)	0.19 (0.11, 0.28)	0.758
SaO_2, %	89.00 (87.10, 91.60)	88.75 (85.70, 93.43)	0.644
sST2, ng/mL	40.50 (14.71, 145.16)	69.15 (12.38, 124.35)	0.599

The data are shown as n (%), mean ± SD, and median (interquartile range). PH, pulmonary hypertension; BSA, body surface area; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second; DLCO, diffusing capacity of the lung for carbon monoxide; LDH, lactate dehydrogenase; CRP, C-reactive protein; NK, natural killer; SaO_2, arterial oxygen saturation; sST2, soluble suppression of tumorigenicity-2.

function and enhances profibrogenic cytokine production, resulting in the initiation and progression of pulmonary fibrosis (7). It has been reported from *in vitro* research that sST2 gene expression increases after profibrogenic stimuli (29,30). A further clinical study has confirmed that the serum sST2 concentrations in patients with pulmonary fibrosis are elevated, especially in AE-IPF (11). In accordance with this study, the sST2 levels were higher in the IPF patients compared with the healthy group in our study. However, no statistical difference in sST2 was found between the survival and non-survival groups and sST2 could not predict all-cause death in the IPF patients. Event-free survival time was significantly shorter for patients of the non-survival group, and event-free survival time proved to be an independent predictor of the all-cause death. Therefore, we further investigated the relationship between sST2 and the event-free survival, and as we expected, the sST2 level was significantly higher in the event group. Above all, sST2 was an independent predictor of event-free survival. Therefore, although the sST2 level could not directly predict all-cause death in IPF patients, a higher serum level of sST2 predicted more deterioration and poor outcomes in the IPF patients. The possible explanation is that the mechanism of all-cause death in IPF patients is complex, not only involving progression of the disease but also deterioration that is mainly caused by aggravation of inflammation and progression of fibrosis. Taken together, for the first time our study has elucidated the exact relationship between sST2 and the prognosis of IPF for better evaluation of the outcomes of these patients. In addition, more subgroups were formed by the combination of the two different independent predictors to provide more clues to evaluating the outcomes of IPF patients in clinical practice.

PH is a well-established complication in IPF patients and associated with significant morbidity and reduced survival, especially for severe PH (31-34). PH due to IPF belongs to Group 3 PH defined as mean pulmonary artery pressure (mPAP) ≥25 mmHg with pulmonary vascular resistance ≥3 Woods units measured by right heart catheterization (RHC). Because RHC is not generally recommended for Group 3 PH according to the latest guidelines (35), the indices of echocardiography were used to define PH in our study. sST2 proved to be an important predictor of PH in a previous study (36). Therefore, we focused on further studying whether the prognostic impaction of sST2 on the IPF was related to the existence of PH. The NT-pro-BNP level represents myocardial dysfunction and provides prognostic information at the time of diagnosis or during follow-up of pulmonary arterial hypertension (PAH) (37-39). Our results showed that NT-pro-BNP was significantly higher in the PH group, which is consistent with the study of PAH (36,40). Nevertheless, no difference was found in sST2 or survival time and event-free survival time between the groups. Moreover, no linear relationship was found between sST2 and NT-pro-BNP in the IPF patients, whereas in the previous study of PAH, contrary to our result, both NT-pro-BNP and sST2 proved to be prognostically valuable. The possible explanation is that, unlike PAH, the hemodynamic deterioration was relatively mild for most of the IPF-related PH in the patients enrolled in our study. All our results suggested that the possible mechanism for the prognostic effect of sST2 on the outcomes of IPF maybe not necessarily involve the development of the PH but is mainly caused by the progression of fibrosis.

Study limitations

The major limitation of this study is that the patient sample size was relatively small and obtained from a single center and thus should be further validated in a larger group of subjects from different regions. Secondly, we were unable to measure the level of ligand IL-33, which may offer extra mechanistic insights into our present results. Moreover, the IPF-related PH patients were not defined by RHC, which may affect the accuracy of the results in terms of the relationship between sST2 and PH.

Conclusions

IPF stands out as a subset of progressive fibrosing interstitial lung diseases resulting in progressive deterioration and poor prognosis. Our study for the first time reported that the sST2 level obtained during the first diagnosis can be used to well estimate outcomes in IPF.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-3215/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-3215/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3215/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of the Nanjing Drum Tower Hospital (No. 2021-390-01). Informed consent was taken from all the patients.

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