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Serum stratifin measurement is useful for evaluating disease severity and outcomes in patients with acute exacerbation of interstitial lung disease: a retrospective study

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Abstract

Background Serum levels of stratifin (SFN), a member of the 14-3-3 protein family, increase in patients with drug-induced lung injury associated with diffuse alveolar damage. Therefore, we hypothesised that SFN levels would be higher in those experiencing acute exacerbation of interstitial lung disease (AE-ILD). A secondary analysis was also planned to determine whether SFN levels could discriminate survival in those with AE.

Methods Thirty-two patients with clinically stable ILD (CS-ILD) and 22 patients with AE-ILD were examined to assess whether high serum SFN levels were associated with AE-ILD and whether SFN levels reflected disease severity or prognosis in patients with AE-ILD.

Results Serum SFN levels were higher in the AE-ILD group than in the CS-ILD group (8.4 ± 7.6 vs. 1.3 ± 1.2 ng/mL, $p < 0.001$). The cut-off value of the serum SFN concentration for predicting 90-day and 1-year survival was 6.6 ng/mL. SFN levels were higher in patients who died within 90 days and 1 year than in patients who survived beyond these time points (13.5 ± 8.7 vs. 5.6 ± 5.3 ng/mL; $p = 0.011$ and 13.1 ± 7.5 vs. 3.1 ± 1.9 ng/mL; $p < 0.001$, respectively) in the AE-ILD group. When this cut-off value was used, the 90-day and 1-year survival rates were significantly better in the population below the cut-off value than in those above the cut-off value ($p = 0.0017$ vs. $p < 0.0001$).

Conclusions High serum SFN levels are associated with AE-ILD and can discriminate survival in patients with AE-ILD.

Keywords Stratifin, Acute exacerbations, Interstitial lung disease, Biomarker

Background

Acute exacerbation (AE) in idiopathic pulmonary fibrosis (IPF) is defined as acute, clinically significant respiratory deterioration characterised by evidence of new widespread alveolar abnormalities [1]. AE is a severe complication of IPF, with a mortality rate of approximately 50% (median survival time: 3–4 months) [2]. Although AE was first identified in patients with IPF, physicians also often observed it in patients with other types of fibrotic interstitial lung disease (ILD). AE occurs in all fibrotic ILDs, including IPF, with a lower frequency in other

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fibrotic ILDs [3–5]. Nevertheless, AE has a similar clinical significance across all types of fibrotic ILDs [5]. Disease behaviour of progressive pulmonary fibrosis (PPF) is defined according the presence of at least two of the following three criteria: worsening respiratory symptoms, pathophysiological decline in lung function, and radiological progression in patients with ILD other than IPF over the course of the past year [3, 4]. AE is the most critical clinical event in PPF [4–6].

Severe forms of AE in interstitial lung disease (AE-ILD) are characterised by pathologically diffuse alveolar damage (DAD) and radiologically bilateral ground-glass opacity or consolidation on high-resolution computed tomography (HRCT) [1]. Although physicians struggle to establish a definitive diagnosis of DAD without pathological findings, patients with DAD pattern-associated lung diseases according to HRCT findings are usually treated with a high dose of steroids or immunosuppressive drugs in a clinical setting. Currently, levels of type II alveolar epithelium-related surfactant protein (SP)-A, SP-D, and Krebs von den Lungen-6 (KL-6) are widely used as biomarkers for detecting interstitial pneumonia [7, 8]. However, these biomarkers are neither specific for AE nor correlated with the severity of lung injury [9]. Therefore, a biomarker that can specifically detect AE-ILD, evaluate the severity of AE-ILD, and ideally predict patient survival is needed.

Stratifin (SFN) is a member of the 14-3-3 protein family and is originally identified as a p53-inducible gene responsive to DNA-damaging agents [10]. SFN is expressed in all eukaryotic cells and plays a critical role in signal transduction pathways and cell cycle regulation [11]. SFN is expressed permanently and abundantly in the skin and oesophageal tissues. In addition, SFN is expressed in various human cancer tissues, and SFN upregulation is a key event during the malignant progression of lung adenocarcinoma. In the lung, SFN is weakly expressed in the normal alveolar epithelium [12]. We previously found higher serum levels of SFN in patients with drug-induced lung injury associated with radiological DAD patterns than in patients with other pathological types of ILD and controls [13]. This indicated that SFN is strongly associated with DAD and suggested that SFN could be associated with AE-ILD [13].

In this study, we hypothesised that SFN levels would be high in those experiencing AE-ILD, and secondary analysis was planned to determine whether SFN levels could discriminate survival in patients with AE.

Methods

Study design and patient selection

The retrospective research protocol regarding ILD biomarkers was approved by the Human Ethics Committee of Chiba University Hospital (approval numbers: 2083 and 2265) and was conducted according to the tenets of the amended Declaration of Helsinki. We obtained written informed consent in the outpatient department.

For the control group, 32 patients with clinically stable ILD (CS-ILD) who did not receive any treatment for ILD, such as antifibrotic agents or corticosteroids, were recruited from the outpatient department. CS-ILD was defined as no obvious clinical deterioration within 3 months. We could not obtain written informed consent from all AE-ILD patients because it was difficult to fully explain the research plan owing to their poor general condition. As a result, only 22 patients who were hospitalised for AE-ILD at our department between 2013 and 2021 were recruited.

Our previous research focused on drug-induced lung injury, including drug-induced ILD exacerbations, showed that serum SFN levels are useful for detecting DAD patterns in chest images in drug-induced lung injury. Thus, we examined serum SFN levels in AE-ILD patients. The present study included four patients previously diagnosed with ILD and developed drug-induced ILD exacerbations. Regarding their underlying ILD, two patients had IPF, one patient had nonspecific interstitial pneumonia, and the other patient had connective tissue disease-associated ILD.

AE-ILD

AE-ILD, including AE-IPF, was diagnosed according to the definitions proposed by the American Thoracic Society [1]. The criteria were as follows: previous or concurrent diagnosis of fibrotic ILD; acute worsening or development of dyspnoea within one month; HRCT images with new bilateral ground-glass opacities and consolidation superimposed on a background pattern consistent with fibrotic ILD; and deterioration not fully explained by cardiac failure or fluid overload. Fibrotic ILDs other than IPF included pleuroparenchymal fibroelastosis, nonspecific interstitial pneumonia, connective tissue disease-associated ILD, chronic hypersensitivity pneumonitis, and unclassifiable interstitial lung disease. Respiratory physicians and radiologists classified the fibrotic ILD phenotype. Pathology-confirmed diagnosis was not applicable.

Measurement of serum SFN by ELISA

Serum SFN levels were measured using an in-house enzyme-linked immunosorbent assay (ELISA) method with two commercially available anti-SFN mouse monoclonal antibodies, as reported previously [13]. This ELISA was validated with reference to the Japanese Guidelines on Bioanalytical Method Validation (Ligand Binding Assay) in Pharmaceutical Development (Japanese

Ministry of Health, Labour and Welfare). The "Guideline on Bioanalytical Method (Ligand Binding Assay) Validation in Pharmaceutical Development" was used (PSFB/ELD Notification No. 0401–1, 1 April 2014, Ministry of Health, Labour and Welfare, Japan). Serum SFN levels were measured using samples from patients with AE-ILD collected at the time of diagnosis before starting steroid and other treatments.

Data collection

Patient characteristics were retrieved from medical records. These included age, sex, body mass index (BMI), serum lactate dehydrogenase (LDH), C-reactive protein (CRP), KL-6, SP-A, SP-D, brain natriuretic peptide (BNP), D-dimer, and SFN levels. The data on pulmonary function (% forced vital capacity and % diffusing capacity of lung for carbon monoxide) were obtained at a clinically stable state within 6 months before AE. Clinical characteristics, including the oxygen saturation/fraction of inspired oxygen (S/F) ratio, were evaluated in patients with AE-ILD and CS-ILD.

Eight patients with AE-ILD died within 90 days, and 3 patients subsequently died within 1 year. The clinical characteristics of the patients in the AE-ILD group were compared between those who survived more than 90 days and those who died within 90 days. Similarly, the characteristics of patients who survived for more than 1 year and those who died within 1 year were evaluated.

ROC curve and Kaplan–Meier analysis

Serum SFN levels were compared between the AE-ILD and CS-ILD groups; those who survived more than 90 days and who died within 90 days; and those who survived more than 1 year and who died within 1 year in the AE-ILD group. The relationships between serum SFN levels and the aforementioned parameters were analysed, after which the cut-off value of SFN for predicting survival for more than 90 days and 1 year was calculated using receiver operating characteristic (ROC) curve analysis. Similarly, the cutoff values of LDH, CRP, BNP, and the S/F ratio to predict survival after 90 days and 1 year were calculated. Clinical data were compared between the survival and nonsurvival groups at 90 days and 1 year.

Kaplan–Meier analysis was conducted to determine whether the cut-off value could distinguish between survivors and nonsurvivors at 90 days and 1 year. Baseline disease severity; any treatment for lung fibrosis; comorbidities such as cardiovascular complications, diabetes, hypertension, and hyperlipidaemia for which any medical treatment had been needed; and dust exposure history could be potential confounding factors that may affect SFN levels. Therefore, it was also examined whether these potential confounding factors influenced serum SFN levels.

Statistical analysis

Continuous variables are expressed as the mean ± SD. The Mann–Whitney U test or Fisher's exact test was performed to compare two unpaired groups. Correlations between the serum parameters were assessed using Pearson's product-moment correlation coefficient. Youden's index was calculated, and receiver operating characteristic (ROC) curve analysis was performed to determine the cut-off value with the best sensitivity and specificity for predicting mortality at 90 days or 1 year. The patients were divided into two groups based on the cut-off values, and Kaplan–Meier curves and log-rank tests were performed to compare the two groups. A p value of < 0.05 was considered to indicate significance. All statistical analyses were performed using the JMP statistical software, version 14.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

CS-ILD and AE-ILD

This cohort included 54 eligible patients: 32 patients with CS-ILD and 22 patients with AE-ILD. Among them, 21 patients with CS-ILD and 12 patients with AE-ILD had a diagnosis of IPF (Table 1). Table 2 summarises the characteristics of the patients in the CS-ILD and AE-ILD groups. LDH, CRP, and D-dimer levels were higher, whereas the S/F ratio was lower in the AE-ILD group than in the CS-ILD group. Serum SFN levels were higher in the AE-ILD group than in the CS-ILD group ($p < 0.001$) (Fig. 1). Among the 32 patients with CS-ILD, 21 patients had IPF, and 11 patients had non-IPF. The mean serum SFN levels in this group were 1.3 ± 1.2 ng/mL for IPF patients and 1.3 ± 1.3 ng/

Table 1 Classification of patients with interstitial lung diseases

	IPF	NSIP	PPFE	CTD-ILD	CHP	UC-ILD
CS-ILD, n	21	2	1	4	1	3
AE-ILD, n	12	2	3	4	1	0

CS clinically stable, *AE* acute exacerbation, *ILD* interstitial lung disease, *IPF* idiopathic pulmonary fibrosis, *NSIP* nonspecific interstitial pneumonia, *PPFE* pleuroparenchymal fibroelastosis, *CTD-ILD* connective tissue disease-associated interstitial lung disease, *CHP* chronic hypersensitivity pneumonitis, *UC-ILD* unclassifiable-interstitial lung disease

Table 2 Comparison of characteristics between patients with CS-ILD and with AE-ILD

	CS-ILD ($n=32$)	AE-ILD ($n=22$)	*p* Value
Age (years)	71.6 ± 4.9	70.8 ± 8.7	0.791
Sex (male/female)	23/9	19/3	0.208
BMI (kg/m^2)	23.2 ± 2.8	21.8 ± 3.2	0.161
LDH (IU/L)	251.0 ± 16.7	393.0 ± 124.0	< 0.001
CRP (mg/dL)	0.2 ± 0.3	7.3 ± 8.3	< 0.001
KL- 6 (U/mL)	1086.2 ± 783.2	1726.8 ± 1096.0	0.013
SP-A (ng/mL)	81.6 ± 42.2	111.0 ± 55.2	0.140
SP-D (ng/mL)	328.1 ± 267.6	578.7 ± 363.6	0.005
BNP (pg/mL)	36.9 ± 36.9	111.7 ± 144.9	0.031
D-dimer (μg/mL)	0.6 ± 0.7	6.3 ± 16.9	< 0.001
SpO_2/FiO_2 ratio	452.4 ± 26.2	332.6 ± 103.0	< 0.001
Serum stratifin (ng/mL)	1.3 ± 1.2	8.4 ± 7.6	< 0.001

Variables are shown as the mean ± SD

BMI body mass index, *LDH* lactate dehydrogenase, *CRP* C-reactive protein, *KL-6* Krebs von den Lungen-6 (reference: < 500 U/mL), *SP-A* surfactant protein-A (reference: < 43.8 ng/mL), *SP-D* surfactant protein-D (reference: < 110.0 ng/mL), *BNP* brain natriuretic peptide

mL for non-IPF patients, with no significant difference ($p=0.7$). Among the 22 patients with AE-ILD, 12 patients had IPF, whereas 10 patients had non-IPF. This group had markedly higher mean serum SFN levels, measuring 8.4 ± 8.2 ng/mL for the IPF patients and 8.5 ± 7.2 ng/mL for the non-IPF patients. However, similar to the CS-ILD group, there was no significant difference observed in SFN levels between IPF and non-IPF patients with AE-ILD ($p=0.7$).

Correlation between serum SFN concentration and other parameters

As shown in Table 3, LDH, CRP, and BNP levels were positively correlated with serum SFN levels (all $p<0.001$), whereas the S/F ratio was negatively correlated ($p<0.001$) in all patients. In the CS-ILD group, CRP, KL-6, and D-dimer levels were positively correlated with serum SFN levels ($p=0.001$, $p=0.001$, and $p=0.029$, respectively). In the AE-ILD group, only BNP was positively correlated with serum SFN levels ($p=0.021$), and the S/F ratio was negatively correlated ($p=0.014$). These results indicated that KL-6, SP-A and SP-D, which are recognised as ILD-related serum biomarkers, may not be appropriate markers for AE-ILD, although serum SFN levels can reflect DAD in patients with AE-ILD.

Survival after 90 days and 1 year in patients with AE-ILD

Eight patients with AE-ILD died within 90 days, and three patients subsequently died within 1 year. Table 4 presents the clinical characteristics of the patients who survived more than 90 days and those who died within 90 days in the AE-ILD group. The CRP and BNP levels were higher, and the KL-6 level and S/F ratio were lower in patients who died within 90 days. The serum SFN level was higher in patients who died within 90 days than in patients who survived for more than 90 days ($p=0.011$) (Fig. 2).

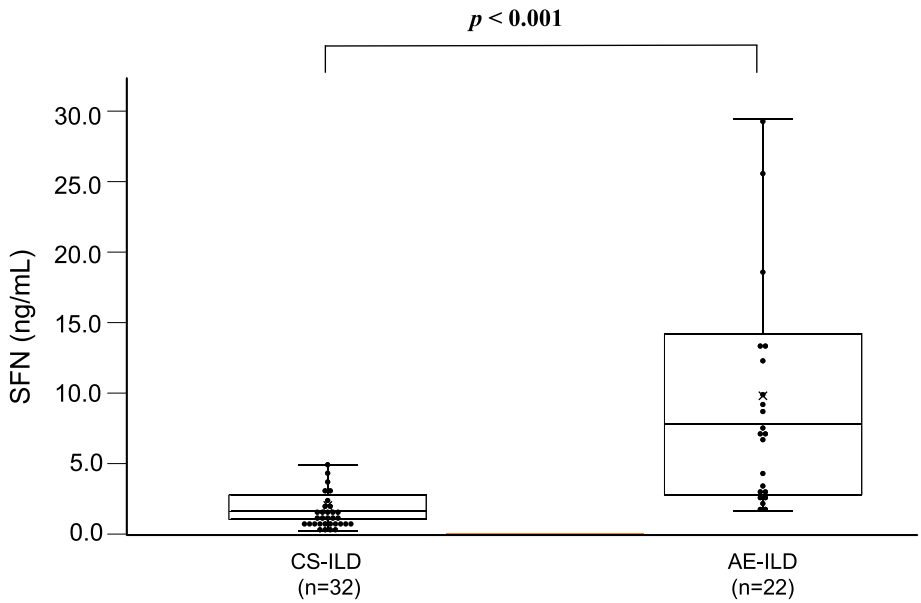

Fig. 1 Serum levels of stratifin (SFN). The values in patients with clinically stable interstitial lung disease (CS-ILD) and acute exacerbations of interstitial lung disease (AE-ILD) are shown. Each dot represents an individual in each group

Table 3 Correlation coefficients between serum stratifin levels and other parameters

	All patients	*p* Value	CS-ILD	*p* Value	AE-ILD	*p* Value
LDH (IU/L)	0.517	< 0.001	0.290	0.107	0.346	0.115
CRP (mg/dL)	0.531	< 0.001	0.597	0.001	0.303	0.171
KL- 6 (U/mL)	0.223	0.106	0.602	0.001	-0.048	0.833
SP-A (ng/mL)	0.238	0.205	0.482	0.037	0.107	0.704
SP-D (ng/mL)	0.282	0.462	0.317	0.088	0.055	0.965
BNP (pg/mL)	0.571	< 0.001	0.110	0.600	0.512	0.021
D-dimer (μg/mL)	0.095	0.537	0.438	0.029	-0.107	0.652
SpO_2/FiO_2 ratio	-0.691	< 0.001	-0.311	0.084	-0.514	0.014

LDH lactate dehydrogenase, *CRP* C-reactive protein, *KL-6* Krebs von den Lungen-6 (reference: < 500 U/mL), *SP-A* surfactant protein-A (reference: < 43.8 ng/mL), *SP-D* surfactant protein-D (reference: < 110.0 ng/mL), *BNP* brain natriuretic peptide

Table 4 Comparison of characteristics between patients who survived and died beyond 90 days in the AE-ILD group

	Surviving over 90 days ($n=14$)	Death within 90 days ($n=8$)	*p* Value
Age (years)	68.3 ± 8.8	75.4 ± 6.7	0.115
Sex (male/female)	12/2	7/1	0.906
BMI (kg/m^2)	22.4 ± 2.4	20.6 ± 4.4	0.179
LDH (IU/L)	360.8 ± 135.3	449.5 ± 79.7	0.024
CRP (mg/dL)	3.4 ± 3.6	9.8 ± 3.5	0.013
KL-6 (U/mL)	2067.1 ± 1230.0	1131.4 ± 392.9	0.041
SP-A (ng/mL)	119.4 ± 61.6	92.9 ± 39.2	0.624
SP-D (ng/mL)	590.5 ± 388.2	557.0 ± 347.6	0.880
BNP (pg/mL)	43.9 ± 44.8	213.3 ± 184.9	0.008
D-dimer (μg/mL)	2.2 ± 2.1	14.0 ± 28.1	0.096
FVC (L)	2.2 ± 0.9	1.9 ± 0.5	0.461
%DLCO (%)	59.6 ± 21.2	57.2 ± 19.6	0.782
SpO_2/FiO_2 ratio	380.8 ± 70.2	248.3 ± 99.5	0.002
Serum stratifin (ng/mL)	5.6 ± 5.3	13.5 ± 8.7	0.011

Variables are shown as the mean ± SD

BMI body mass index, *LDH* lactate dehydrogenase, *CRP* C-reactive protein, *KL-6* Krebs von den Lungen-6 (reference: < 500 U/mL), *SP-A* surfactant protein-A (reference: < 43.8 ng/mL), *SP-D* surfactant protein-D (reference: < 110.0 ng/mL), *BNP* brain natriuretic peptide, *FVC* forced vital capacity, *DLCO* diffusing capacity of the lungs for carbon monoxide

Table 5 shows the characteristics of the patients who survived for > 1 year and those who died within 1 year in the AE-ILD group. LDH, CRP and BNP levels were higher, and the S/F ratio was lower in those who died within 1 year. The serum SFN level was significantly higher in patients who died within 1 year than in patients who survived > 1 year ($p < 0.001$) (Fig. 3).

Ability of serum SFN levels and other variables to predict 90-day and 1-year survival

The optimal cut-off value of serum SFN levels for predicting 90-day and 1-year survival was determined by ROC curve analysis. For the prediction of 90-day survival, the optimal cut-off value was 6.6 ng/mL (sensitivity, 100%; specificity, 71.4%; area under the curve (AUC), 0.84). However, that for predicting 1-year survival was also 6.6 ng/mL (sensitivity, 100%; specificity, 90.1%; AUC, 0.98).

For the 90-day survival rate, the area under the ROC curve was 0.79 (sensitivity, 87.5%; specificity, 71.4%) for LDH, 0.83 (sensitivity, 75.0%; specificity, 85.7%) for CRP, 0.89 (sensitivity, 87.5%; specificity, 75.0%) for BNP, and 0.90 (sensitivity, 75.0%; specificity, 100%) for the S/F ratio (Fig. 4). For the 1-year survival rates, the values were 0.77% (sensitivity, 90.9%; specificity, 63.6%) for LDH, 0.76% (sensitivity, 81.8%; specificity, 63.6%) for CRP, 0.88% (sensitivity, 90.0%; specificity, 70.0%) for BNP, and 0.90% (sensitivity, 90.9%; specificity, 72.7%) for the S/F ratio (Fig. 5).

Kaplan–Meier analysis

To confirm the SFN cut-off levels in the ROC curve to discriminate between survivors and nonsurvivors, the patients were divided into two subgroups with low SFN (< 6.6 ng/mL) and high SFN (≥ 6.6 ng/mL). No patients in the low SFN subgroup died within 90 days or 1 year. The 90-day survival rate was significantly higher in the low SFN subgroup ($p = 0.0017$) (Fig. 6). Similarly, the 1-year survival rate was significantly higher in the low SFN subgroup ($p < 0.0001$) (Fig. 7).

Previously set SFN cut-off value

In our previous study on drug-induced lung injury associated with radiological DAD patterns, a serum SFN level of 3.6 ng/mL was established as the optimal cut-off value for discriminating the DAD pattern in chest images [13]. Thus, we used a serum SFN level of 3.6 ng/mL to discriminate AE-ILD from CS-ILD in the present study. In the CS-ILD group, 31 patients were included in the low SFN subgroup, and only 1 patient was included in the high SFN subgroup. In the AE-ILD

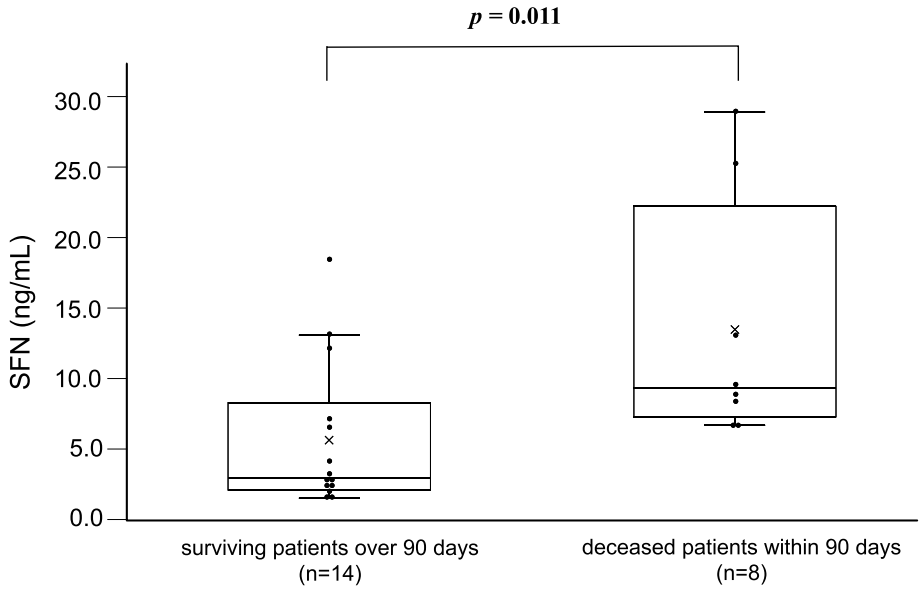

Fig. 2 Serum levels of stratifin in patients who survived and died after > 90 days. Each dot represents an individual in each group

Table 5 Comparison of characteristics between patients who survived and died beyond 1 year in the AE-ILD group

	Surviving over 1 year ($n=11$)	Dying within 1 year ($n=11$)	*p* Value
Age (years)	68.9 ± 9.3	72.8 ± 7.9	0.510
Sex (male/female)	9/2	10/1	0.531
BMI (kg/m^2)	21.9 ± 2.4	21.7 ± 4.0	0.888
LDH (IU/L)	353.4 ± 150.0	432.7 ± 79.3	0.033
CRP (mg/dL)	3.6 ± 4.0	11.0 ± 9.9	0.042
KL- 6 (U/mL)	1772.7 ± 1183.5	1680.9 ± 1056.1	0.577
SP-A (ng/mL)	102.0 ± 62.1	118.0 ± 51.5	0.418
SP-D (ng/mL)	510.1 ± 382.8	639.7 ± 356.8	0.386
BNP (pg/mL)	39.2 ± 47.6	184.1 ± 174.4	0.004
D-dimer (μg/mL)	2.3 ± 2.4	10.3 ± 23.7	0.273
FVC (L)	2.3 ± 0.9	1.8 ± 0.5	0.242
% DLCO (%)	67.0 ± 16.9	47.8 ± 20.0	0.088
SpO_2/FiO_2 ratio	398.5 ± 65.5	266.6 ± 91.8	0.002
Serum stratifin (ng/mL)	3.1 ± 1.9	13.7 ± 7.5	< 0.001

Variables are shown as the mean ± SD

BMI body mass index, *LDH* lactate dehydrogenase, *CRP* C-reactive protein, *KL-6* Krebs von den Lungen-6 (reference: < 500 U/mL), *SP-A* surfactant protein-A (reference: < 43.8 ng/mL), *SP-D* surfactant protein-D (reference: < 110.0 ng/mL), *BNP* brain natriuretic peptide, *FVC* forced vital capacity, *DLCO* diffusing capacity of the lungs for carbon monoxide

group, 8 patients were included in the low SFN subgroup, and 14 patients were included in the high SFN subgroup. The sensitivity for AE diagnosis was 63.6%, and the specificity was 96.9%.

Confounding factors possibly related to SFN levels in the AE-ILD group

In the AE-ILD group, 12 patients had a history of lung fibrosis treatment before AE, whereas 10 patients were treatment naive. Antifibrotic drugs were started in three patients, prednisolone in seven patients, and a combination of both in two patients. No significant difference in SFN levels was observed according to treatment type for lung fibrosis (9.9 ± 9.0 ng/mL vs. 6.7 ± 5.4 ng/mL; $p=0.575$). Similarly, no significant difference in SFN levels was observed between 17 patients with comorbidities and 5 patients without comorbidities (8.7 ± 8.0 ng/mL vs. 7.3 ± 6.6 ng/mL; $p=0.784$). Further, there was also no significant difference in SFN levels between 5 patients with a history of dust exposure and 17 patients without a history of dust exposure (4.1 ± 2.5 ng/mL vs. 9.7 ± 8.2 ng/mL; $p=0.196$). Overall, no obvious confounding factors affecting SFN levels were detected, although this study included a limited number of patients.

Confounding factors possibly related to SFN levels in the CS-ILD group

The potential confounding factors influencing SFN value, such as baseline therapies, comorbidities and disease severity, were examined in the CS-ILD group. In the 32 patients with CS-ILD, the serum SFN levels did not differ between the 21 patients with and 11 without comorbidities (1.3 ± 1.2 ng/mL vs. 1.3 ± 1.2 ng/mL, $p=0.858$). For disease severity evaluated using the GAP score, 14 patients had stage I disease, 17 patients

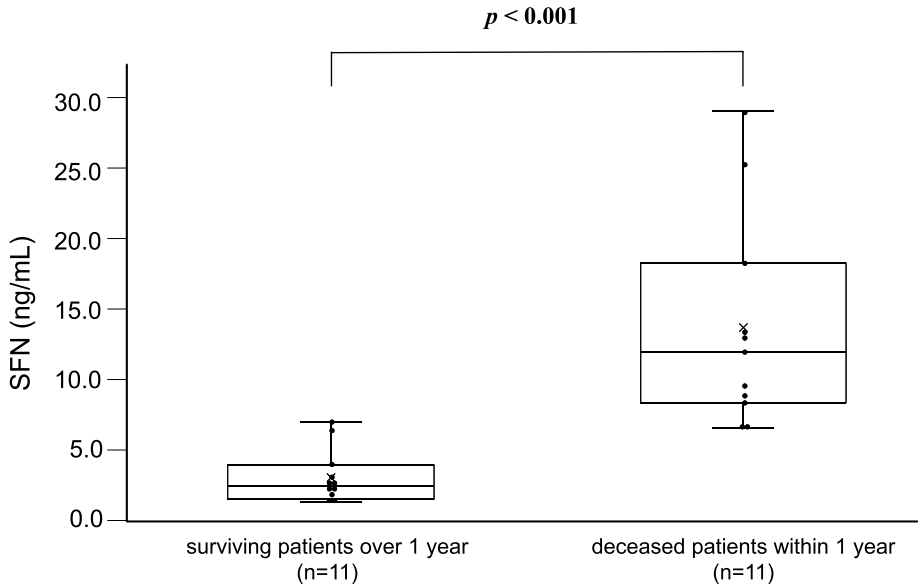

Fig. 3 Serum levels of stratifin in patients who survived and died after > 1 year. Each dot represents an individual in each group

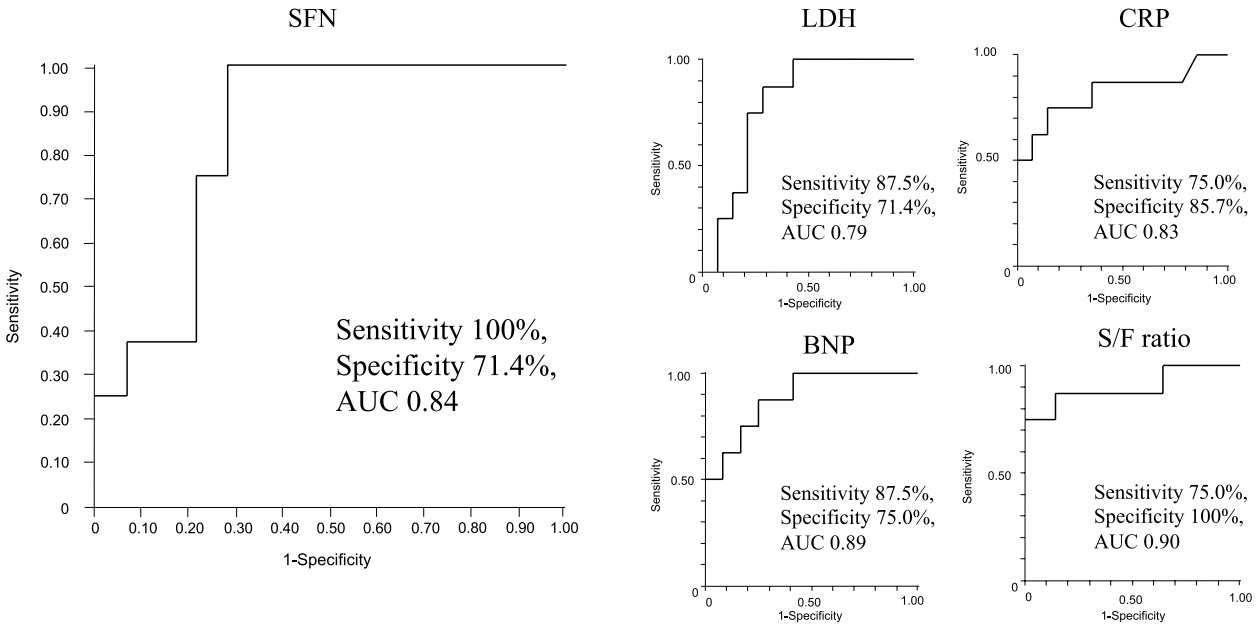

Fig. 4 Receiver operating characteristic (ROC) curves for optimal cut-off values for predicting 90-day survival. The values for serum SFN, lactate dehydrogenase (LDH), C-reactive protein (CRP), and brain natriuretic peptide (BNP) and the SpO2/FiO2 (S/F) ratio are shown

had stage II disease, and 1 patient had stage III disease. Serum SFN levels did not significantly differ according to the GAP score in these three groups (1.2 ± 1.2 ng/mL, 1.4 ± 1.2 ng/mL, and 1.0 ng/mL, respectively, $p = 0.748$) [14]. Similarly, no significant difference was detected between 6 patients with a history of dust exposure (1.5 ± 1.5 ng/mL) and 26 patients without a history of dust exposure (1.3 ± 1.2 ng/mL) ($p = 0.646$).

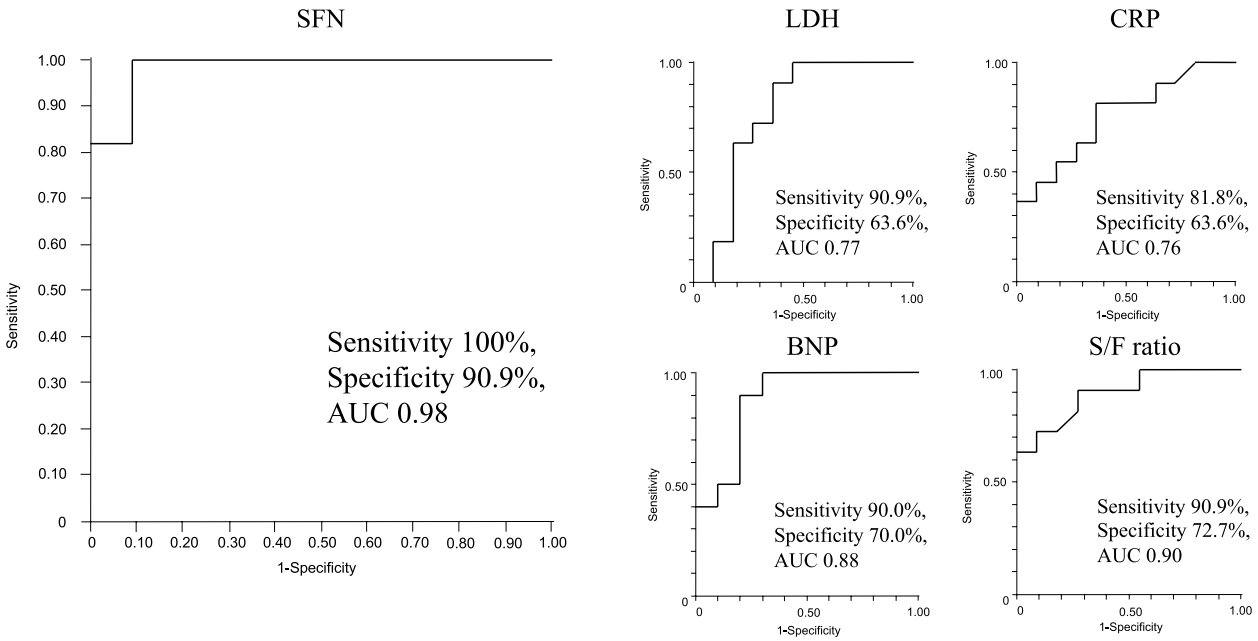

Fig. 5 ROC curves for the optimal cut-off values for predicting 1-year survival. The values for serum SFN, LDH, CRP, and BNP and the S/F ratio are shown

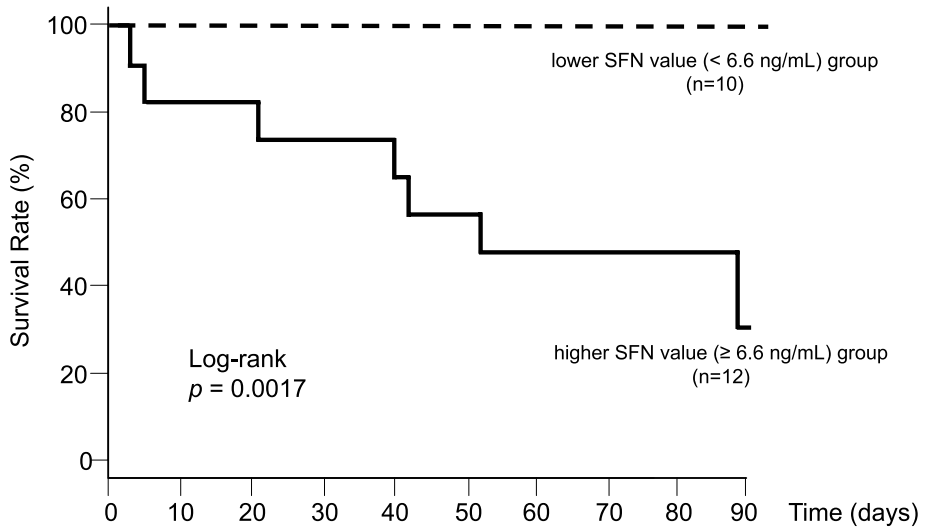

Fig. 6 Ninety-day survival rate. The 90-day survival rate is significantly higher in patients whose SFN values are below the cut off (< 6.6 ng/mL) than in those whose SFN values are above the cut off (≥ 6.6 ng/mL) ($p = 0.0017$). SFN: stratifin

Discussion

Severe forms of AE-ILD are characterised by pathological DAD and radiological DAD patterns, such as bilateral ground-glass opacity or consolidation on HRCT. However, in clinical settings, DAD cannot always be pathologically confirmed, and clinicians often rely on the integration of subjective and objective findings and radiological images and laboratory data, including any biomarkers, to start appropriate treatment for AE-ILD. Newly developed biomarkers that can specifically detect AE-ILD are needed.

In the present study, the serum SFN concentration was higher in patients with either IPF or non-IPF-related AE-ILD than in those with CS-ILD. When the cut-off levels of SFN was set at 6.6 ng/mL, no patients in the low SFN subgroup died within 90 days or 1 year. We previously

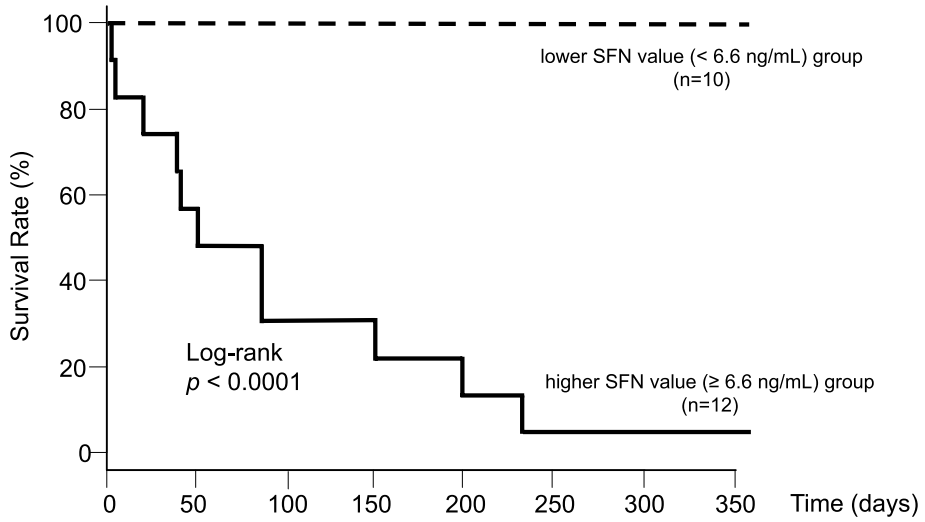

Fig. 7 One-year survival rate. The 1-year survival rate is significantly higher in patients whose SFN values are below the cut-off (< 6.6 ng/mL) than in those whose SFN values are above the cut-off (≥ 6.6 ng/mL) ($p < 0.0001$). SFN: stratifin

reported that the serum levels of SFN were significantly higher in patients with drug-induced lung injury associated with radiological DAD patterns [13]. In the current study, we demonstrated that serum levels of SFN were associated with AE-ILD.

SFN (14-3-3σ) belongs to the 14-3-3 protein family, together with six other isoforms, namely, β, γ, ε, ζ, η, and τ [15]. This family constitutes a group of highly evolutionarily conserved proteins with a molecular mass of 25–30 kDa that are expressed in all eukaryotes and are involved in the modulation of various cellular processes by binding to phosphorylated proteins [11]. Although most 14-3-3 isoforms are ubiquitously expressed in various normal tissues, SFN is specifically expressed in stratified epithelium [16]. In lung tissues, SFN is weakly expressed in the normal respiratory alveolar epithelium, and SFN expression is reportedly associated with lung cancer [12, 17]. We recently reported that the levels of SFN in serum and bronchoalveolar lavage fluid (BALF) were associated with DAD patterns in patients with drug-induced ILD. Importantly, we found that serum SFN levels were specifically increased in patients with DAD and could thus be used as a biomarker of drug-induced ILD [13]. The performance of SFN as a biomarker in drug-induced DAD was superior to that of existing biomarkers such as KL-6, SP-D and SP-A, which were expressed in type II alveolar epithelial cells.

Although the intracellular function and biological activity of SFN have been investigated, no definitive conclusions have been reached. SFN expression is induced in a p53-dependent manner in response to DNA damage, and the progression of the G2/M phase of the cell cycle is arrested [18]. Alveolar epithelial damage may trigger an increase in the extracellular SFN concentration via the p53 pathway, thereby increasing the serum SFN concentration. We previously reported that BALF and serum SFN concentrations were correlated [13]. SFN is considered a marker of the differentiation of epidermal keratinocytes. SFN released from keratinocytes is thought to be associated with skin wound healing and the suppression of skin fibrosis [19]. Alveolar epithelium-produced SFN maybe associated with pulmonary damage and healing. In the present study, serum LDH levels were positively correlated with serum SFN levels in all patients with ILD. LDH is ubiquitously present in all tissues and is known to be a nonspecific marker of tissue turnover. AE-ILD is often triggered by infection, and LDH usually increases as a result of lung damage, whereas serum SFN is not elevated in infectious pneumonia or collagen disease [13]. Thus, SFN is more specific to DAD than are other biomarkers, including LDH.

BNP levels showed a positive correlation with serum SFN levels in AE-ILD patients. AE-ILD is usually associated with the right heart load, and as a result, BNP levels increase. The evaluation of comorbidities did not include chronic heart failure in this study. In the normal clinical setting at our institute, echocardiographs are performed to differentiate heart failure in patients with high BNP levels. High BNP levels were observed within 90 days or 1 year in the nonsurvivor subgroup, who had a poor prognosis. The area under the ROC curve for predicting 90-day survival was higher for BNP than that for SFN (0.89 vs. 0.84). This suggests that the right heart load, as indicated by serum BNP levels, is associated

with short-term survival within 90 days in patients with AE-ILD. However, the area under the ROC curve for the prediction of 1-year survival was lower for BNP than for SFN (0.88 vs. 0.98). This suggested that the degree of lung parenchymal damage, reflecting the range of DAD in the lung area, could influence the long-term survival at 1 year in patients with AE-ILD.

In the present study, patients with serum SFN levels above the cut-off value (≥6.6 ng/mL) had a poorer prognosis. We divided the patients with AE-ILD into two subgroups according to their serum SFN levels to determine whether the levels established using the ROC curve reflected the prognosis of patients with AE-ILD. We examined 90-day and 1-year survival and found that the subgroup with an SFN above the cut-off value had a significantly worse prognosis ($p=0.0017$ and $p<0.0001$, respectively). AE is a clinically significant event that is correlated with increased mortality. Despite extensive research efforts, the exact pathogenesis of AE remains unclear to date, and AE remains the leading cause of death in patients with ILD in Japan [20]. Serum biomarkers that are useful for predicting the risk of AEs in patients with ILD have been explored, and KL-6, interleukin-6, monomeric periostin, decorin, and syndecan-4 have been proposed as candidate predictive biomarkers [20–25]. Some biomarkers are related to AE and prognosis; however, a biomarker associated with DAD has not been developed. Many studies used inflammatory cytokines as biomarkers for AE-ILD [26–28]. However, SFN, derived from the respiratory alveolar epithelium, might be more relevant to alveolar epithelial damage because it is only mildly elevated in cases of lung infection. KL-6 is also a protein derived from the alveolar epithelium, but it is not useful for prognostic determination [9]. The present study demonstrated that the serum levels of SFN are reflective of the disease state and could be associated with the disease course and prognosis of AE-ILD. However, whether serum SFN levels were a prognostic biomarker could not be determined because of the small sample size, retrospective nature of the analysis, insufficient evidence to indicate a direct relationship between SFN and diagnosis or outcomes, lack of a validation arm, lack of a replication cohort, and lack of correction for potential confounding factors as described below.

This preliminary study has several limitations, including its small sample size. This study also lacked a validation arm and a replication cohort. Additionally, we could not obtain consecutive samples in the AE-ILD patients. The difficulty in obtaining informed consent owing to the poor general condition of the AE-ILD patients may have also contributed to the limited samples obtained. Therefore, selection bias and small sample size pose potential limitations. When comparing clinical data between patients with AE-ILD and CS-ILD, the levels of LDH, CRP, KL-6, SP-D, BNP, and D-dimer, in addition to those of SFN, were higher in the AE-ILD group. Each of these biomarkers reflect specific aspects of AE-ILD. However, it was challenging to treat these variables as independent variables in the multivariate analysis, yielding no statistically significant power.

For clinical significance, larger samples and longitudinal studies, ideally with paired samples at stable and AE states, are needed. Consequently, this study lacked evidence for a direct relationship between SFN and diagnosis and outcomes. Although we evaluated confounding factors such as lung fibrosis treatments, including antifibrotic drugs, disease severity, comorbidities, and dust exposure history, we could not identify any obvious confounding factor affecting SFN levels. This study provided insufficient evidence to indicate a direct relationship between SFN levels and diagnosis or outcomes because of the limited number of patients. Future studies are needed to confirm the pathophysiological significance of serum SFN levels.

Conclusions

The serum levels of SFN are significantly higher in patients with AE-ILD than in patients with CS-ILD. This suggests that SFN can be associated with AE-ILD, as we also previously reported that the serum SFN level reflects the DAD in drug-induced lung injury, the main pathology of AE-ILD. In addition, serum SFN concentration, at a cut-off of 6.6 ng/mL, is associated with the 90-day and 1-year survival rates in patients with AE-ILD.

Abbreviations

AE	Acute exacerbation
AE-ILD	Acute exacerbation of interstitial lung disease
BALF	Bronchoalveolar lavage fluid
BNP	Brain natriuretic peptide
CRP	C-reactive protein
CS-ILD	Clinically stable interstitial lung disease
DAD	Diffuse alveolar damage
DLCO	Diffusing capacity of lung for carbon monoxide
HRCT	High-resolution computed tomography
IPF	Idiopathic pulmonary fibrosis
KL-6	Krebs von den Lungen-6
LDH	Lactate dehydrogenase
PPF	Progressive pulmonary fibrosis
ROC	Receiver operating characteristic
SFN	Stratifin
SP	Surfactant protein

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Authors' contributions

NS was a major contributor to the writing of the manuscript. NS, DI, TK and MA collected specimens and analysed the patient data. MA, NA, SM and YS

measured SFN levels in samples using ELISA. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets analysed during the current study are not publicly available due protection of personal information but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The clinical trial research protocol was approved by the Human Ethics Committee of Chiba University Hospital (approval number: 2083 and 2265) and complied with the Declaration of Helsinki Ethical Principles for medical research involving human subjects. We obtained written informed consent from all the patients.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests. NA and YS are inventors of patents related to stratifin involved in the diagnosis of ILD as filed by the National Institute of Health Science.

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