Upregulation of S100 Calcium-Binding Protein A9 levels in the lungs of patients with idiopathic pulmonary fibrosis

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Abstract

Background: Neutrophilic inflammation is a predominant characteristic of idiopathic pulmonary fibrosis (IPF). S100 Calcium-Binding Protein A9 (S100A9) is a neutrophil-derived protein and is involved in the development of neutrophil-related chronic inflammatory disorders. However, the role of S100A9 in IPF has not been evaluated.

Methods : S100A9 concentrations were measured by ELISA in the BAL fluid obtained from NCs (n = 33) and patients with IPF (n = 87), NSIP (n = 22), HP (n = 19), or sarcoidosis (n = 10).

Results: The S100A9 levels in BALF were significantly higher in patients with IPF than in those with NC (0.4 [0.18–0.9] vs. 0 [0–0.5] ng/mL, p < 0.001), HP (0.19 [0.07–0.33] ng/mL, p = 0.043), or sarcoidosis (0.06 [0–0.11] ng/mL, p < 0.001) patients. A S100A9 level of 0.093 ng/mL had discriminating powers of 78.79% for specificity and 81.61% for sensitivity between IPF patients and NCs. S100A9 levels were also correlated with neutrophil numbers (r = 0.356, p = 0.0007) and S100A9 was expressed on neutrophils and macrophages in the BALF of IPF patients. Patients with S100A9 levels above 0.5535 ng/mL or a neutrophil percentage above 49.09% (n = 43) had significantly lower survival rates than those with S100A9 levels at or below 0.5535 ng/mL and a neutrophil percentage at or below 49.09% (n = 41) (HR, 9.28; p = 0.0004).

Conclusion: S100A9 may participate in the development and progression of IPF. The levels of S100A9 in BALF may be a surrogate marker for diagnosing IPF and predicting its prognosis.

Introduction

Idiopathic interstitial pneumonia (IIP) is a group of lung diseases whose etiology is unknown. IIP is characterized by an accumulation of inflammatory cells in the pulmonary parenchyma and interstitium that leads to fibrosis. Idiopathic pulmonary fibrosis (IPF), the most common form of IIP, results in gradual deterioration with a diverse clinical course [1, 2]. The poor prognosis of IPF is due to alveolar epithelial injury and abnormal repair mechanisms that ultimately develop into irreversible pulmonary fibrosis [3]. Abnormalities in multiple biological pathways lead to processes related to the pathogenesis of IPF, and affect inflammation and wound repair. The processes and factors include the clotting cascade, oxidant–antioxidant pathways, apoptosis, inflammatory cytokines, angiogenesis, vascular remodeling, growth factors, surfactants, and matrix regulatory factors [4, 5]. Among the inflammatory patterns, neutrophilic inflammation is predominant in bronchoalveolar lavage fluid (BALF) of IPF patients [6–8] and is related to early mortality in these patients [6, 9]. Levels of the neutrophil chemoattractant CXCL8 are higher in the BALF and serum of patients with IPF [10]. In addition, levels of the alveolar epithelial marker cytokeratin 19 are correlated with neutrophil infiltration, suggesting a relationship between epithelial injury and neutrophilic inflammation [11].

Neutrophilic inflammation was recently revealed as being related to fibrosis. In neutrophilic inflammation, neutrophil granules release neutrophil elastase (NE) and matrix-degrading proteins such as matrix

metalloproteinases (MMPs), which are the main tissue destructive agents and are heavily involved in matrix degradation [12]. NE increases in the lungs of IPF patients [8] and regulates the degradation of the extracellular matrix (ECM) into collagens I, II, III, IV, fibronectin, laminin, and elastin. It also induces fibroblast proliferation and myofibroblast differentiation [13, 14].

S100A9 is one of the main mediators of neutrophils and macrophages and is now considered a damage-associated molecular pattern protein (DAMP) because it exists intracellularly and is released upon activation or damage under conditions of cellular stress [15]. S100A9 has the potential to cause fibroblast proliferation [16], and to upregulate collagen type III α-smooth muscle actin and the receptor for advanced glycation end-product expression (RAGE) [17]. Higher concentrations of S100A9 have been reported in the BALF of patients with IPF than in that from patients with other ILDs, including sarcoidosis, non-specific interstitial pneumonia (NSIP), and pulmonary fibrosis associated with connective tissue diseases that have a potential biomarker [18, 19]. However, previous studies have provided only limited information since they had a small number of subjects. Therefore, we measured the concentrations of S100A9 in BALF to evaluate the association between S100A9 and the development of IPF. We also investigated the clinical impact of IPF, particularly the long-term survival rate in a relatively large number of subjects and those with ILDs.

Materials And Methods

Study subjects

Lung tissues and BALF from study subjects were obtained from the biobank of Soonchunhyang University Hospital, Bucheon, Korea (Schbc-biobank-18101601-14-01). The study protocol was approved by the Ethics Committee of Soonchunhyang University Hospital (Schbc-medicine-2018-10). Informed written consent for study participation was obtained from the subjects, and a sample donation was obtained from each subject. All subjects were examined by physicians and underwent a chest X-ray, high-resolution chest computed tomography (HRCT), and pulmonary function tests. There was no evidence of any underlying collagen vascular diseases in IPF patients according to their laboratory results and clinical symptoms. The diagnostic criteria for IPF, hypersensitivity pneumonitis (HP), NSIP, and sarcoidosis were established based on an international consensus statement [20–23]. IPF was diagnosed by the presence of usual interstitial pneumonia (UIP) patterns in the pathological specimens (surgical IPF) and/or by HRCT in patients who did not undergo surgical lung biopsy (clinical IPF). Two pathologists examined each slide independently after being informed of the subjects' sex, age, and HCRT results. Pathological recognition of the NSIP pattern included two major aspects: (1) exclusion of other patterns of interstitial lung diseases, and (2) categorization of the histological features according to the ATS/ERS 2002 classification [23, 24] and the modified histological definition of the NSIP pattern [25]. HP was diagnosed by the presence of clinical symptoms compatible with non-necrotizing granulomatous bronchiolocentric pneumonitis [20]. The diagnosis of sarcoidosis was based on histological evidence of non-caseating granuloma and compatible clinical images [22]. HP and sarcoidosis were diagnosed after excluding other diseases with similar histological profiles. Biopsy tissues were subjected to acid-fast bacillus and Gömöri

methenamine silver staining to verify the absence of microorganisms and fungi. The serial diffusing capacity of lungs for carbon monoxide (DLCO) and forced vital capacity (FVC) were measured, and the annual rate of decline was estimated as follows: (last FVC [or DLCO] − baseline FVC [or DLCO])/baseline FVC [or DLCO]/follow-up years. Normal controls (NC) exhibited no respiratory symptoms as determined by a screening questionnaire and had a predicted forced expiratory volume at 1 second (FEV1) and FVC > 80% and normal chest radiograms.

Bronchoalveolar lavage fluids procedure

The bronchial alveolar lavage fluid (BALF) procedure was performed in lung segments that were not under immunosuppressive therapy, were in the right middle lobe of the NCs, and exhibited the greatest disease involvement when HRCT was done, as described previously [26]. A cytocentrifuge was used to prepare the cells, which were then mounted on slides and stained with Diff-Quik stain. A hemocytometer was used to differentially count the cells to a total count of 500 cells. The supernatants were isolated using centrifugation (500 × g, 5 min) and stored at − 80 °C.

Enzyme-linked immunosorbent assay (ELISA) of S100A9 in BALF

The S100A9 level in the supernatants was measured using an ELISA (MyBioSource, San Diego, CA, USA) according to the manufacturer's recommendations. The lower limit of detection was 0.1 ng/mL, and values below this limit were set to 0. The inter- and intra-assay coefficients of variation were below 15%.

Immunofluorescence stain of S100A9 in bronchial alveolar lavage fluid cells and lung tissues

Tissue sections were deparaffinized and rehydrolyzed, and BALF cell slides were fixed with 0.4% cold paraformaldehyde for 30 minutes at room temperature. The sections were then incubated overnight at 4 °C with macrophage markers, including monoclonal anti-human S100A9 monoclonal mouse antibody (1:200 dilution; Novus Biological, Littleton, CA, USA), polyclonal anti-human α-SMA antibody (1:200 dilution; Abcam, Cambridge, MA, USA), and monoclonal goat anti-CD163 antibody (1:200 dilution, Hycult Biotech, Uden, PB, Netherlands). The sections were also incubated with monoclonal rabbit anti-neutrophil elastase antibody (1:100 dilution; Abcam, Cambridge, MA, UK) as a neutrophil marker. Tissue sections were deparaffinized and rehydrolyzed, and BALF cell slides were fixed with 0.4% cold paraformaldehyde for 30 minutes at room temperature. The sections were incubated for 1 hour in an Fc receptor blocking agent (FC blocker, Innovex Biosciences, Richmond, CA, USA) containing 5% bovine serum albumin (BSA) to block non-specific binding. The sections were then incubated overnight at 4 °C with macrophage markers, including monoclonal anti-human S100A9 monoclonal mouse antibody (1:200 dilution; Novus Biological, Littleton, CA, USA), polyclonal anti-human α-SMA antibody (1:200 dilution; Abcam, Cambridge, MA, USA), and monoclonal goat anti-CD163 antibody (1:200 dilution, Hycult Biotech, Uden, PB, Netherlands). The sections were also incubated with monoclonal rabbit anti-neutrophil elastase antibody (1:100 dilution; Abcam, Cambridge, MA, UK) as a neutrophil marker. Additional details are provided in the

on-line supplement. After washing three times with Tris Buffered Saline (TBS), the slides were incubated for 1 hour at room temperature with the following fluorescent secondary antibodies: anti-rabbit IgG H&L (FITC) (1:1,000 dilution; Abcam, Cambridge, MA, UK), anti-mouse IgG H&L (PE) (1:1,000 dilution; Abcam, Cambridge, MA, UK), and anti-goat IgG H&L (PE) (1:1,000 dilution; Abcam, Cambridge, MA, UK). After washing in TBS, the slides were incubated for 3 minutes at room temperature with 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, St. Louis, USA) and observed under a confocal laser scanning microscope (LSM 510 META, Zeiss, Jena, Germany).

Statistical analysis

The Kruskal–Wallis test and the post-hoc Mann–Whitney U test were used to compare the S100A9 levels between groups. Correlations between the S100A9 levels and other parameters were analyzed using Spearman's correlation coefficient. The data are presented as medians in the 25% and 75% quartiles for variables with a skewed distribution, or as a mean ± standard error of the mean for variables with a normal distribution. Receiver operating characteristic (ROC) analysis was performed, and the area under the ROC curve (AUC) and cutoff values were determined using MedCalc statistical software. Optimal cutoff levels of S100A9 and the neutrophil percentage were calculated using Cutoff Finder [27]. Survival rates were estimated using the Kaplan–Meier method and compared using a log-rank test. The data were analyzed using SPSS software v. 20.0. Values of $p < 0.05$ were considered statistically significant.

Results

Study group patient demographic characteristics

BALFs were obtained from patients with IPF (n = 87) and those with ILDs including NSIP (n = 22), HP (n = 19), and sarcoidosis (n = 10). BALFs were also obtained from NC patients (n = 33). The clinical characteristics of all patients are summarized in Table 1. The BALFs of patients with IPF and ILDs had significantly higher numbers of macrophages, neutrophils, lymphocytes, and eosinophils compared to NC patients. They also had lower FVC and FEV1 values than those of NCs ($p < 0.05$).

S100A9 levels in BALFs of patients with IPF or other ILDs and normal controls

The levels of S100A9 were significantly higher in BALF from patients with IPF than in that from NC patients (IPF, 0.4 [0.18–0.9] ng/mL vs. 0 [0–0.5] ng/mL, $p < 0.001$; HP, 0.19 [0.07–0.33] ng/mL, $p = 0.043$; sarcoidosis, 0.06 [0–0.11] ng/mL, $p < 0.001$). However, the S100A9 levels were not different from those with NSIP (0.28 [0.07–0.52] ng/mL, $p = 0.1933$) (Fig. 1A). The S100A9 levels significantly correlated with neutrophil counts in the BALF of patients with IPF (n = 87, $r = 0.356$, $p = 0.0007$) (Fig. 1B). The ROC curve showed a clear distinction between the IPF patients and the NCs (AUC = 0.833, Fig. 1C). A cutoff level of 0.093 ng/mL S100A9, determined from the ROC curve, showed a specificity of 78.79% and a sensitivity of 81.61% for differentiating IPF patients from NCs. A cutoff level of 0.0279 ng/mL S100A9 exhibited a specificity of 72.73% and a sensitivity of 76.47% for distinguishing patients with IPF from those with other ILDs (AUC = 0.735, Fig. 1D).

Survival rates and clinical features in relation to S100A9 levels and percentage of neutrophils in BALF from IPF patients

Eighty-four patients with IPF were followed for 1 to 10 years. Cutoff values for the S100A9 level and the neutrophil percentage were chosen, and the subjects were divided into two groups. With a cutoff value of 0.5536 ng/mL, the survival rate was significantly lower in the group with a S100A9 level above 0.5536 ng/mL (n = 35) than in the group that had a S100A9 level at or below 0.5536 ng/mL (n = 49; hazard ratio (HR), 3.62, 95% confidence interval (CI), 1.13–11.63; p = 0.021, Fig. 2A). With a cutoff value of 49.09% neutrophils, the survival rate was significantly lower in the group with a neutrophil percentage above 49.09% (n = 17) compared with the group with a neutrophil percentage at or below 49.09% (n = 67; HR, 3.11; 95% CI, 1.5–11.26; p = 0.003, Fig. 2B). A combined analysis of the neutrophil percentage and the S100A9 levels in BALF was performed with respect to the survival rate. Patients with an S100A9 level above 0.5536 ng/mL or a neutrophil percentage above 49.09% (n = 43) had a significantly lower survival rate than those with S100A9 levels at or below 0.5536 ng/mL and a neutrophil percentage at or below 49.09% (n = 41; HR, 9.28; 95% CI, 2.08–41.26; p = 0.0004, Fig. 2C). Patients that had S100A9 levels above 0.5536 ng/mL (n = 35) had a significantly higher total cell count and neutrophil count in BALF than those that had S100A9 levels at or below 0.5536 ng/mL (n = 49; p = 0.0004 and p < 0.0002, respectively; Supplemental Table 1). Patients with or a neutrophil percentage above 49.09% (n = 17) tended to have a higher neutrophil count in BALF than those with a neutrophil percentage at or below 49.09% (n = 67; p = 0.079, Supplemental Table 2). In the combination analysis (Table 2), males were predominant and the BALF total cell counts were higher in the group that had S100A9 levels above 0.5535 ng/mL or a neutrophil percentage above 49.09% (n = 43) than in those with S100A9 levels at or below 0.5535 ng/mL and a neutrophil percentage at or below 49.09% (n = 41) (p = 0.02). Otherwise, there were no differences in age, BMI, smoking status, GAP stage, or lung function.

S100A9 levels in lung tissues and BALF cells of patients with idiopathic pulmonary fibrosis measured by immunofluorescence staining

To confirm S100A9 expression in the lungs of patients with IPF, S100A9/α-SMA double immunofluorescence staining was performed on lung tissues of three IPF patients and three controls. In the control lung tissues, perivascular and peribronchial areas were stained by both α-SMA and S100A9. In the IPF lung tissues, α-SMA was robustly expressed by interstitial fibroblasts, most of which also expressed S100A9 (Fig. 3). To confirm S100A9 expression in the BALF cells of IPF patients, S100A9 and CD163 double immunofluorescence staining was performed. In the cells of these patients, CD163 was robustly expressed by macrophages, most of which also expressed S100A9 (Fig. 3).

Discussion

In this study, we demonstrated that S100A9 levels were significantly higher in BALF from patients with IPF than in BALF from NC subjects, or those with other ILDs. Additionally, S100A9 level of 0.093 ng/ml and 0.0279 ng/mL exhibited high sensitivity and specificity for diagnosing IPF and for differential

diagnosis of other ILDs, respectively. Over the past decade, higher concentrations of S100A9 in BALF from IPF patients have been reported compared to patients with other ILDs [18, 19, 28, 29]. However, these reports have only come from a relatively small number of patients. The concentrations of S100A9 in BALF were inversely correlated with impairments in lung function, as indicated by reduced forced vital capacity and diffusing capacity of carbon monoxide [19]. Certain concentrations of S100A9 also showed sufficient specificity and sensitivity to distinguish IPF from NSIP and CVD-IP [28]. Recently, Bennett et al. reported that higher concentrations of S100A9 were related to a more advanced IPF condition, lower lung function values, a shorter distance walked in the 6-minte walk test, and BALF neutrophilia in 30 patients with IPF [30]. It is important to re-evaluate the clinical implications of these parameters in a larger number of IPF patients. The present study demonstrated that the level of S100A9 in BALF is a surrogate biomarker for survival chances. A cutoff value of 0.5536 ng/mL divided IPF patients into short- and long-term survival groups with a hazard ratio of 3.62.

S100A9 is a small calcium-binding protein released by stressed cells that are undergoing necrosis [15]. S100A9 acts as an endogenous danger signal that accelerates and exacerbates the neutrophilic response in non-infectious inflammation via enhanced chemotaxis of neutrophils and macrophages and modulation of their functions [31, 32]. Because of the predominance of neutrophilic inflammation in the lungs of IPF patients [6, 7], S100A9 had been presumed to be responsible for the neutrophilic inflammation of IPF. This hypothesis was well demonstrated in the present study by a good correlation between the two parameters. Additionally, a cutoff value of 49.09% for neutrophils in BALF divided patients into short- and long-term survival groups with a hazard ratio of 3.11. Interestingly, when a combined analysis of neutrophil percentage and S100A9 levels was performed, patients with S100A9 levels above 0.5536 ng/mL and a neutrophil percentage above 49.09% had a significantly lower survival rate than those with S100A9 levels at or below 0.5536 and neutrophil percentage at or below 49.09%, with a hazard ratio of 9.28, three times higher than the hazard ratio of the BALF S100A9 level or the neutrophil percentage individually. This indicates that both high levels of S100A9 or high percentages of neutrophils may be related to early mortality in IPF.

Thee S100A9 and neutrophils in BALF are known to be involved in the pathogenesis of inflammatory conditions such as asthma [33, 34] and COPD [35], and there is also ample evidence of S100A9 having fibrotic effects. The process of fibrosis has been shown to be mediated by combinatorial signaling pathways that involve components of the TGF-β/CTGF pathway, and also signaling events induced by epidermal growth factor (EGF) and Insulin like growth factor 2 (IGF-2) activated receptors [36]. S100A9 also stimulates the proliferation of fibroblasts, albeit at higher concentrations than the former factors [3, 5]. In addition, S100A9 induces cellular communication network factor 2 (CCN2) mRNA and protein, the latter of which stimulates fibroblast growth and myofibroblast differentiation [36] via Toll-like receptor 4 (TLR4) and RAGE [37]. Type 1 alveolar epithelial (AT1) cells [38] and type 2 alveolar epithelial (AT2) cells [39] express high levels of RAGE in lung [40]. S100A9 enhances the basal migratory motility of fibrocytes [41] and promotes lung fibroblast activation, resulting in the expression of collagen type III and α-smooth muscle actin via the RAGE pathway [17], and RAGE levels are increased in IPF [42]. In addition, TLR4 is strongly expressed in parenchymal fibroblasts and infiltrating cells located at fibrotic loci [43]. Thus,

patients with high levels of S100A9 may have a more rapid fibrotic process that leads to a lower survival rate.

One limitation of this study is that it did not measure other mediators of IPF besides neutrophils. In this study, some patients with low levels of S100A9 and a high neutrophil percentage also showed early mortality. This indicates that mediators other than S100A9 produced from neutrophils may contribute to early mortality in IPF. Neutrophil elastase degrades various ECM components [13] and leads to fibroblast proliferation and myofibroblast differentiation in a SMAD-dependent but TGF-β-independent fashion [14]. An additional mediator is the neutrophil extracellular trap, a major product of neutrophils that promotes fibrosis via TGF-β1 production and subsequent myofibroblast activation [44]. Thus, these mediators should be evaluated and analyzed for their role in IPF mortality in the future.

Conclusion

To understand the role of S100A9 in neutrophilic inflammation related to IPF, S100A9 concentrations were measured in BALF. The levels of S100A9 were significantly higher in IPF patients than in NC subjects and patients with other ILDs. Furthermore, the levels of S100A9 had good discriminating power for the differential diagnosis of IPF. The survival rates were significantly lower in IPF patients that had higher S100A9 levels and neutrophil percentages than those with lower S100A9 levels and neutrophil percentages. These results suggest that S100A9 participates in the development and progression of IPF, and that the IPF levels in BALF could be used as a surrogate marker for diagnosing IPF and predicting prognosis.

Abbreviations

IPF: Idiopathic pulmonary fibrosis, ECM: extracellular matrix, BAL: bronchoalveolar lavage, NC: normal controls, NSIP: non-specific interstitial pneumonia, HP: hypersensitivity pneumonitis, ROC: receiver operating characteristic

Declarations

Ethics approval and consent to participate

It was writing at the materials and methods sections; as follows: The study protocol was approved by institutional review board (IRB) in the Soonchunhyang University hospital ethics committee (medicine 2018-10).

Consent for publication

All authors have read and approved the submission of manuscript.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Available

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Authors' contributions

JU Lee and CS Park conceived and designed the experiments, JU Lee, RH Kim, JS Park and ES Go performed IF stain, IHC stain and ELISA, CS Park and JS Park provided clinical samples, JS Park, HS Chang and JU Lee performed statistical analysis, Lee JU, HS Chang and CS Park wrote the manuscript.

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Tables

Table 1. Clinical characteristics of study participants who underwent bronchoalveolar lavage

Parameters	NC	IPF	ILDs		
			NSIP	HP	Sarcoidosis
Number of subjects	33	87	22	19	10
Age (year)	55 (35–72)	63.8 (32–86)*	60.1 (39–70)	51.3 (28–70)	43.3 (28–69)
Sex (male/female)	20/13	54/33	9/13	10/9	6/4
Smoking (CS/ES/NS)	2/1/30	38/29/20	2/5/12	3/2/13	3/2/5
FVC (% pred.)	106.1 (87.0–119)	75.0 (63.7–83.0)*	78.0 (66.0–91.8)*	64.5 (57.0–82.5)*	77.0 (65.0–86.0)*
FEV1 (% pred.)	102.1 (88.2–117)	89.0 (77.5–100.5)*	85.0 (73.8–101.3)*	74.5 (64.3–92.0)*	85.0 (64.0–101.0)*
DLCO (% pred.)	NA	64.0 (38.5–72.5)*	76.0 (59.0–92.0)*	67.0 (55.0–90.0)*	75.5 (57.8–84.5)*
dFVC (%/year)	NA	−7.0 (−16.5–0.0)	NA	NA	NA
dDLco (%/year)	NA	-13(-30-0)	-5(-20.5-0)	NA	NA
Follow-up (years)	NA	4.1 (2.1–6.3)	ND	ND	ND
BALF total cells (10^4/mL)	3.42±0.96	5.61±3.71*	6.09±2.74*	6.12±3.79*	4.31±2.92*
Macrophages (10^4/mL)	3.25±0.93	3.43±2.24*	3.16±1.89*	3.19±2*	3.71±2.76*
Neutrophils (10^4/mL)	0.08±0.07	1.66±2.3*	2.44±2.23*	2.7±2.89*	0.37±0.4*
Eosinophils (10^4/mL)	0.02±0.03	0.29±1.1*	0.17±0.27*	0.17±0.21*	0.01±0.01*
Lymphocytes (10^4/mL)	0.07±0.06	0.23±0.25*	0.32±0.29*	0.41±0.43*	0.22±0.18*

IPF, idiopathic pulmonary fibrosis, NSIP, non-specific interstitial fibrosis; HP, hypersensitivity pneumonitis; CS/ES/NS: current smoker/ex-smoker/never smoker; ND, not determined, NA, not applicate; dFVC (%)/year, annual decline rate of forced vital capacity (FVC); dDLco (%/year),annual decline rate of diffusing capacity of the lungs for carbon monoxide (DLco).

Patient characteristics and pulmonary function test results are shown as medians (inter-quartile range). Groups among the normal controls and the IPF, NSIP, HP, and sarcoidosis patients were calculated with a Kruskal–Wallis analysis of variance (ANOVA) with the Mann–Whitney U as the post-hoc test. BALF cell numbers are shown as mean ± standard error of the mean (SEM) and were calculated with a one-way ANOVA and Tukey's honestly significant difference test as the post-hoc test among the five groups. Significance: compared with control, *P < 0.05; compared with IPF, †P < 0.05; compared with NSIP and HP, #P < 0.05.

Table 2. Clinical characteristics of patients with IPF classified according to the levels of S100A9 and percentage of neutrophils in BALF

Parameter	S100A9 > 0.5536 ng/mL or Neu % > 49.09%	S100A9 ≤ 0.5536 ng/mL and Neu % ≤ 49.09%	P value
Number of subjects	43	41	-
Age (years)	65 (56.5–73)	63 (59–68)	0.3423
BMI	23.34 (22.16–24.84)	21.97 (19.77–24.03)	0.0417
Sex (male/female)	22/21	31/10	0.0202
Smoking (CS/ES/NS)	21/12/10	15/17/9	0.3929
Gap score	2 (2–5)	4 (2.5–4.25)	0.6914
FVC (% pred.)	75.86 ± 14.84	77.07 ±17.5	0.5788
FEV1 (% pred.)	89.93 ± 16.44	91.32 ± 19.19	0.7169
DLCO (% pred.)	67.02 ± 23.18	70.25 ± 16.99	0.2102
dFVC (%/year)	–7.74 ± 18.26	–12.33 ± 13.23	0.3590
dDLco (%/year)	–15.1 ± 18.35	–20.85 ± 16.05	0.0586
Follow-up duration (years)	4.06 (2.57-5.94)	4.25 (2.17–7.02)	0.5373
Survival/death	28/15	39/2	0.5603
BAL total cell count (10^4/mL)	6.9 ± 4.59	4.36 ± 1.96	0.0016
Macrophages (10^4/mL)	3.58 ± 2.88	3.31 ± 1.41	0.2808
Neutrophils (10^4/mL)	2.62 ± 2.91	0.71 ± 0.71	3.82E-08
Eosinophils (10^4/mL)	0.44 ± 1.53	0.14 ± 0.25	0.2369
Lymphocytes (10^4/mL)	0.27 ± 0.28	0.21 ± 0.22	0.5357

CS/ES/NS, current smoker/ex-smoker/never smoker; dFVC (%)/year, annual decline rate of forced vital capacity (FVC); dDLco (%/year), annual rate of diffusing capacity of the lungs for carbon monoxide (DLco). Differences in patient characteristics and pulmonary function test results are shown as medians (inter-quartile range). Bronchoalveolar lavage (BAL) cell numbers are shown as mean ± standard error of the mean (SEM).

Supplemental Information Note

Supplemental Figure. S100A9 protein concentrations in BAL fluids from IPF patients according to the GAP stage, smoking, and sex.

Comparisons of S100A9 protein levels according to (A) the GAP stage (B) smoking status, and (C) sex.

Figures

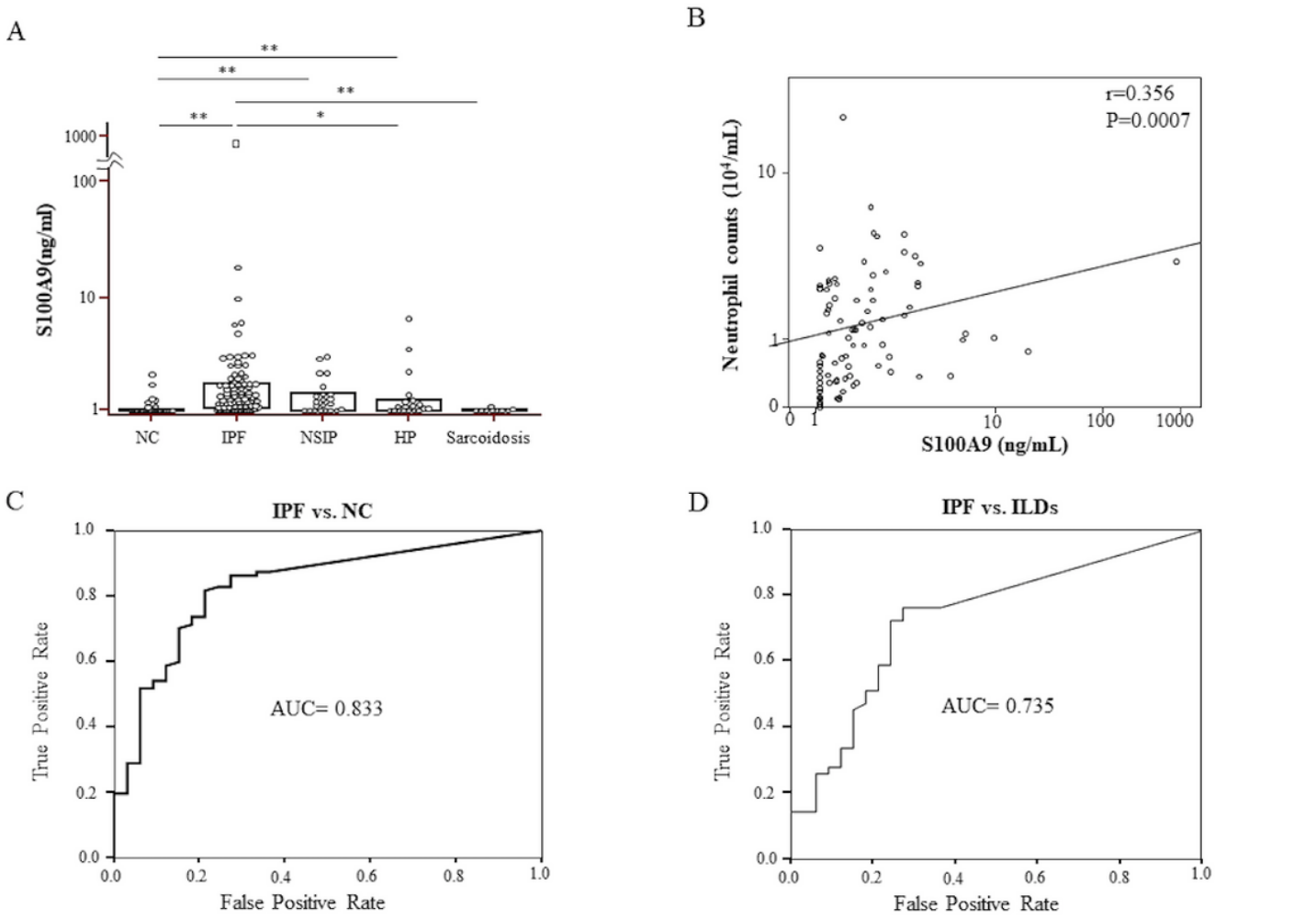

Figure 1

S100A9 concentrations, correlation with neutrophil percentage in BALF, and receiver operating characteristic (ROC) analysis. (A) S100A9 was detected in 12 of 33 normal controls, 76 of 87 IPF patients, 17 of 22 NSIP patients, 15 of 19 HP patients, and six of 10 sarcoidosis patients. The data are presented as medians (inter quartile range). * $P < 0.05$; ** $P < 0.01$. (B) Correlation between S100A9 levels and neutrophil percentages in BALF ($n = 87$, $r = 0.356$, $p = 0.007$). (C) ROC curves of S100A9 concentrations in the IPF and NC groups. A cutoff value of 0.093 ng/mL had an area under the ROC curve (AUC) of 0.833, a specificity of 78.79%, and a sensitivity of 81.61% for differentiating IPF patients from normal controls. (D) A cutoff level of 0.0279 ng/mL S100A9 exhibited a specificity of 72.73% and a sensitivity of 76.47% for distinguishing between IPF patients and those with other interstitial lung diseases (AUC = 0.735).

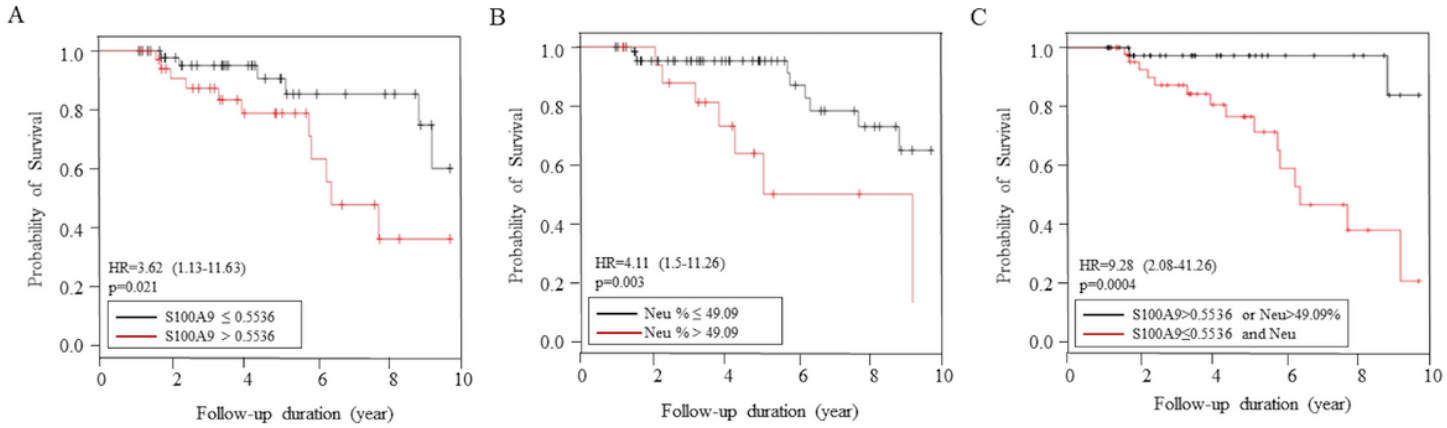

Figure 2

Survival rates in relation to S100A9 levels and neutrophil percentages in BALF from idiopathic pulmonary fibrosis (IPF) patients. A Kaplan–Meier plot was used to analyze 84 subjects with IPF who were followed for one to ten years. (A) Comparison of survival rates of the group with S100A9 levels above 0.5536 ng/ml (n = 49, solid line) and that with S100A9 levels at or below 0.5536 ng/ml (n = 35, dotted line) (HR = 3.62; 95% CI, 1.13–11.63; p = 0.021). (B) Comparison of survival rates of the group with a neutrophil percentage above 49.09% (n = 67, solid line) and that with a neutrophil percentage at or below 49.09% (n = 17, dotted line) (HR = 3.11; 95% CI, 1.5–11.26; p = 0.003). (C) Comparison of survival rates in patients with S100A9 levels above 0.5536 ng/mL (n = 41) or a neutrophil percentage above 49.09% (n = 43, solid line), and those with S100A9 levels at or below 0.5536 ng/mL and a neutrophil percentage at or below 49.09% (n = 41, dotted line) (HR = 9.28; 95% CI, 2.08–41.26; p = 0.0004). Neu %, neutrophil percentage; HR, hazard ratio.

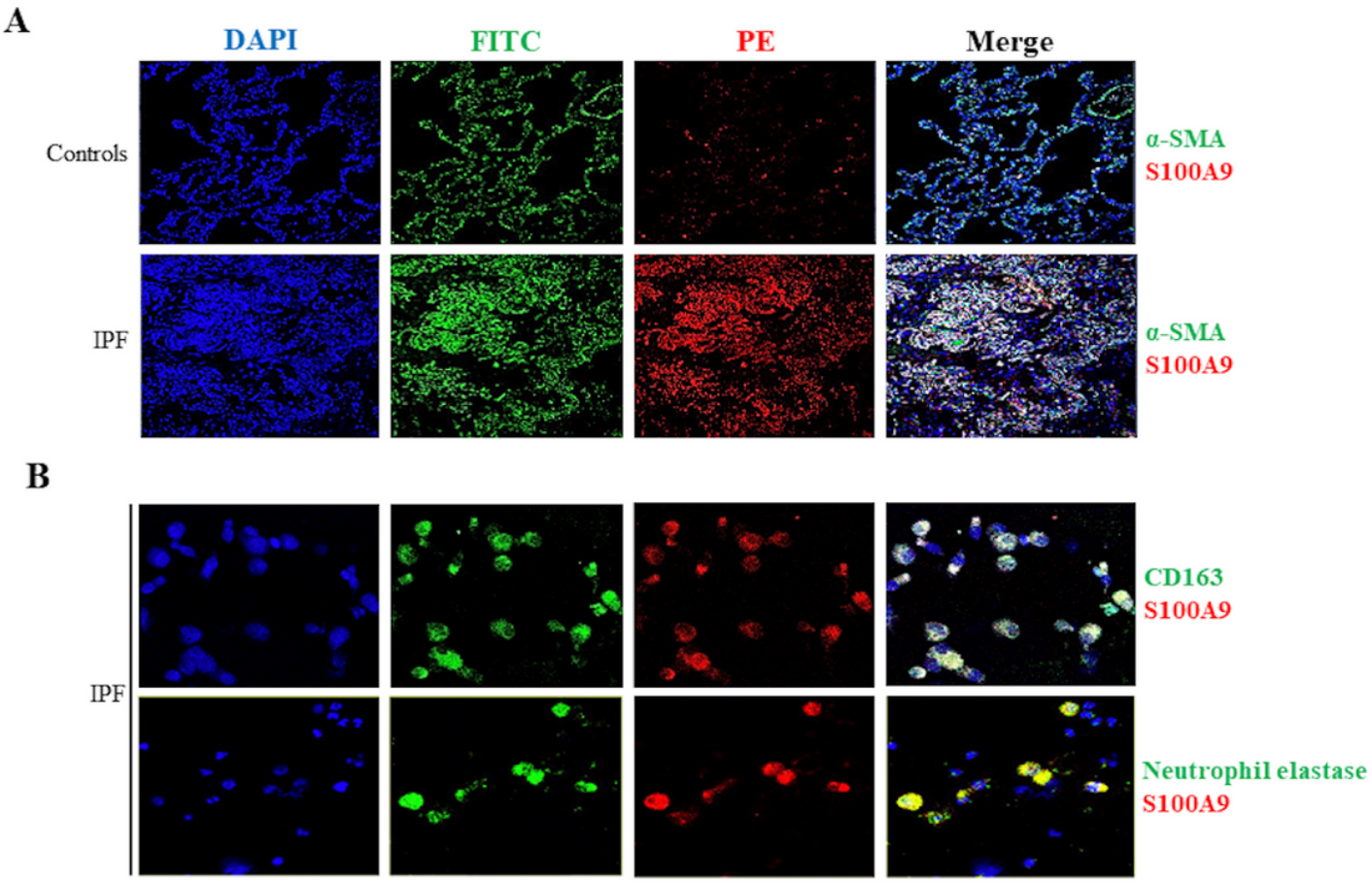

Figure 3

Supplementary Files

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