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Serum surfactant proteins-A and -D as biomarkers in idiopathic pulmonary fibrosis

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Serum surfactant proteins-A and -D as biomarkers in idiopathic pulmonary fibrosis. K.E. Greene, T.E. King Jr, Y. Kuroki, B. Bucher-Bartelson, G.W. Hunninghake, L.S. Newman, H. Nagae, R.J. Mason. ©ERS Journals Ltd 2002.

ABSTRACT: Idiopathic pulmonary fibrosis (IPF) has a high mortality rate, and current therapies are only marginally effective. A serum biomarker that predicts clinical outcome would be useful to stage disease, indicate prognosis and the need for aggressive therapy, and help stratify patients for clinical trials.

The goals of this study were to determine whether serum levels of surfactant protein-A (SP-A) or surfactant protein-D (SP-D) would distinguish between IPF and other types of interstitial lung disease and whether serum SP-A or SP-D levels predict outcome in patients with IPF.

The authors found that serum SP-A and SP-D levels were significantly elevated in patients with IPF and systemic sclerosis compared to sarcoidosis, beryllium disease and normal controls, and that SP-D correlated with radiographic abnormalities in patients with IPF. In addition, the authors found that both serum SP-A and SP-D levels were highly predictive of survival in patients with IPF.

This is the largest North American data set of surfactant protein measurements in idiopathic pulmonary fibrosis and the first report using multivariate analysis comparing serum surfactant proteins-A and -D to other commonly measured predictors of survival in idiopathic pulmonary fibrosis. Based on these results, the authors propose that serum surfactant proteins may prove to be useful biomarkers in patients with idiopathic pulmonary fibrosis.

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Idiopathic pulmonary fibrosis (IPF) carries a 50% 5-yr survival rate [1], and current therapies are only marginally effective in improving pulmonary function or survival time. The pathogenesis of IPF is characterized as excessive wound healing with chronic inflammation, fibroblast proliferation and extracellular matrix production with chronic scarring and honeycomb formation. This fibroproliferative response is uniformly accompanied by type II cell hyperplasia [2]. Surfactant proteins-A (SP-A) and -D (SP-D), produced and secreted by type II cells, can be detected in serum and are elevated in patients with certain inflammatory lung diseases, including IPF [3–9]. Although the exact mechanism for the increase in SP-A and SP-D in the circulation is not known, it is probably a combination of a loss of epithelial integrity due to injury and an increased mass of type II cells due to hyperplasia. Because the concentrations of serum SP-A and SP-D probably vary with disease and lung inflammation, measurement of these two proteins might prove to be useful markers of disease in IPF.

The major goal of this study was to measure serum SP-A and SP-D levels simultaneously in two large cohorts of patients with IPF, and compare these values to patients with other interstitial lung diseases (ILDs). A second goal was to determine whether serum surfactant protein levels predict survival time in patients with IPF. The results demonstrate that serum SP-A and SP-D levels are elevated in patients with IPF and progressive systemic sclerosis (PSS), diseases characterized by type II cell hyperplasia and usual interstitial pneumonia pathology, and are not elevated in the other pulmonary diseases studied with less diffuse parenchymal involvement. In addition, the authors report, in a large number of patients, that serum SP-A and SP-D levels are highly predictive of survival in patients with IPF.

Methods

Study population

Serum samples were collected from 142 patients with IPF, 19 patients with chronic beryllium disease (CBD), 37 patients with PSS and 46 normal volunteers from National Jewish. At the University of Iowa, sera were collected from 68 patients with IPF, 161 patients with sarcoid and 49 normal volunteers. When data were obtained longitudinally on a subject, only the first observation per subject was used in the analyses. This study was performed after approval by the Committee for Research on Human Subjects at the various institutions, and informed consent from all of the patients and the healthy volunteers had been obtained at their respective institutions.

Disease classification

Patients enrolled from Denver. Idiopathic pulmonary fibrosis. The study population consisted of patients and healthy volunteers enrolled in an ILD study supported by a Specialized Centre of Research Grant from the National Institutes of Health. Of the 142 IPF patients included in the study, 105 patients had lung tissue obtained by open lung biopsy, eight had video-assisted thoracoscopic surgery, 17 had transbronchial biopsies and 12 had no biopsy and were determined to have IPF based on clinical and computed tomography scan criteria only. Serum was obtained during the initial visit in 136 patients, at the 1-month visit in two patients, at the 6-month visit in three patients and at the 1-yr visit in one patient. Patients with connective tissue disease, drug or chemical exposures, or other possible aetiologies of ILD such as hypersensitivity pneumonitis, bronchiolitis obliterans, acute interstitial pneumonia, diffuse alveolar damage, bronchiolitis obliterans organizing pneumonia, chronic aspiration, lymphocytic interstitial pneumonia, chronic heart or renal failure, or unclassifiable forms of chronic interstitial pneumonia, were excluded.

Chronic beryllium disease. The diagnosis of CBD was based on the following criteria: 1) history of beryllium exposure; 2) positive bronchoalveolar lavage (BAL) beryllium lymphocyte proliferation test; 3) transbronchial lung biopsy yielding pathological changes consistent with CBD, *i.e.* noncaseating granulomas and/or mononuclear cell infiltrates; and 4) respiratory symptoms, pulmonary function test abnormalities, or chest radiographic small opacities profusion score $\geqslant$1/0 by the International Labour Organization (ILO) classification system. In addition, none of the individuals had other detectable causes of granulomatous inflammation, and all had negative cultures and special stains for acid-fast bacilli, fungi and bacteria.

Systemic sclerosis. All subjects were $\geqslant$18 yrs old and were enrolled as part of a clinical study at the National Jewish Medical Centre. All patients fulfilled the diagnostic criterion of the American Rheumatism Association, namely, the finding of either the sole major criterion, *i.e.* proximal scleroderma (sclerodermatous changes proximal to the metacarpophalangeal or metatarsophalangeal joints, affecting parts of the extremities, face, neck, or trunk), or two or more of the following minor criteria: 1) sclerodactyly; 2) digital pitting scars of the finger tips or loss of substance of the distal finger pad; and 3) bilateral basilar pulmonary fibrosis. For the purpose of this study, pulmonary fibrosis was defined by two or more of the following clinical, radiographical or physiological parameters: "Velcro" rales, increased interstitial markings, reduced total lung capacity (TLC), decreased diffusing capacity, and pressure/volume curve displaced downwards and to the right.

Patients enrolled from Iowa. Idiopathic pulmonary fibrosis. Sixty-eight patients with IPF were included in this study. All of these patients were identified as part of the National Heart, Lung and Blood Institute's (NHLBI) Specialized Centres of Research (SCOR) Programme in Occupational and Immunologic Lung Disease, in which patients with ILD were studied prospectively. The diagnosis of IPF was based on accepted criteria [10], which included either evidence of diffuse parenchymal infiltrates (peripheral and reticular nodular with a lower lobe predominance) on chest radiograph or restrictive lung function with an open lung biopsy demonstrating varied degrees of idiopathic fibrosis and intra-alveolar inflammatory cells. Strict exclusionary criteria were established [10]. Of the 68 patients with IPF, 46 had open lung biopsies, 13 had transbronchial biopsies, and the remaining nine fulfilled all of the clinical criteria required for this diagnosis. Study subjects without open lung biopsies were required to have diffuse parenchymal infiltrates on the chest radiograph, restrictive lung function (TLC <80% predicted), and to meet all of the exclusionary criteria.

Sarcoid. These studies were conducted as a part of a SCOR programme sponsored by the NHLBI. All patients with a diagnosis of sarcoidosis were eligible for entry into the study. The only patients not entered were those who refused to participate in the study. A total of 161 patients with biopsy-proven sarcoidosis were entered into the study. All of the patients exhibited the typical clinical features of sarcoidosis and none had a history of exposure to agents known to cause granulomatous disease [11–13]. In addition, stains and cultures of the biopsy specimens showed no evidence of mycobacterial or fungal infection.

Blood sample collection

Serum samples were collected and stored at -20°C until use.

Table 1. – Demographical data for patients and healthy volunteers

	Denver patients					Iowa patients		
	With ARDS	With CBD	With IPF	With PSS	Healthy volunteers	With IPF	With sarcoid	Healthy volunteers
Subjects n	21	19	142	37	46			
Male	13 (61.9%)	14 (73.7%)	93 (65.5%)	7 (18.9%)	30 (65.2%)	35 (51.5%)	53 (33.1%)	28 (57.1%)
Caucasian	6 (28.6%)	17 (89.5%)	129 (90.9%)	27 (73.0%)	42 (91.3%)	66 (97.1%)	137 (85.1%)	46 (93.9%)
Smoking								
Current		2 (10.5%)	20 (14.1%)	7 (18.9%)	1 (3.5%)	10 (17.2%)	14 (11.2%)	6 (25.0%)
Former		9 (47.4%)	78 (54.9%)	11 (29.7%)	1 (3.5%)	32 (55.2%)	29 (23.2%)	12 (50.0%)
Never		8 (42.1%)	44 (31.0%)	19 (51.4%)	27 (93.1%)	16 (27.6%)	82 (65.6%)	6 (25.0%)
Age yrs mean±SD	42.6±16.5	48.4±8.9	60.0±12.9	49.5±12.7	37.9±8.6	62.5±12.4	45.5±11.6	40.2±10.5

Data are presented as n (%) unless otherwise stated. ARDS: acute respiratory distress syndrome; CBD: chronic beryllium disease; IPF: idiopathic pulmonary fibrosis; PSS: progressive systemic sclerosis.

Enzyme-linked immunosorbent assay for human surfactant protein-A

Human SP-A was measured by an enzyme-linked immunosorbent assay (ELISA) kit, using two monoclonal antibodies provided by the Teijin Institute of Bio-Medicine (Hino, Japan), and the assay was performed by a method based on that of SHIMIZU *et al.* [14]. This assay system was able to detect SP-A at 12.5–100 $ng \cdot mL^{-1}$. All assays were performed in duplicate, and data are expressed as the mean value.

Enzyme-linked immunosorbent assay for human surfactant protein-D

Human SP-D was measured by ELISA, which has been described previously [15]. Recombinant SP-D was used as the standard for this assay, as previously described [16]. The ELISA was based on a sandwich method, using two monoclonal antibodies against human SP-D, 6B2 and 7C6, which were prepared against human SP-D purified from BAL fluids of patients with pulmonary alveolar proteinosis, as previously reported [16]. All assays were performed in duplicate.

Statistical analysis

The Kruskal-Wallis test was used to compare the medians of serum SP-A and SP-D levels across sites (Iowa and Denver) in normal subjects, and then across disease groups within sites. Data from each site were analysed separately due to differences in SP-A values across sites observed in normal subjects. When necessary, Dunn′s nonparametric multiple comparison procedure was used. The relationship between serum SP-A and SP-D levels and other measures of disease severity, were assessed using Spearman′s correlation coefficients. Kaplan-Meier curves were used to describe the survival time in patients with IPF who smoked at the time of the study, never smoked and formerly smoked. In all survival analyses, deaths not due to IPF and transplants were considered censored at time of death or transplant. All covariates in the Cox proportional hazards model were tested to determine whether the proportional hazard assumption was reasonable by including an interaction of time with the covariate of interest. All statistical tests were two-sided and conducted at the 5% level of significance.

Results

Demographical characteristics of the study population

Patients and normal volunteers from two different institutions (National Jewish and University of Iowa) were studied. The demographical characteristics of the normal volunteers and patients from both institutions are outlined in table 1.

Analysis of healthy volunteers' serum surfactant proteins-A and -D

There was a significant difference in the median SP-A level from normal volunteers between study sites, with the median SP-A level being higher in Denver (39.6 $ng \cdot mL^{-1}$) than Iowa (22.2 $ng \cdot mL^{-1}$). There was no significant difference in the median SP-D level from the Iowa volunteers (87.0 $ng \cdot mL^{-1}$) compared to the Denver volunteers (97.3 $ng \cdot mL^{-1}$). Because of the site-to-site differences in SP-A values, however, data from Denver and the University of Iowa were analysed separately, and patients were compared to normal values from that site. The authors also determined that there were no differences in serum SP-A or SP-D levels in normals with storage time, sex, age or smoking (data not shown).

Analysis of interstitial lung disease patients' serum surfactant proteins-A and -D

The serum SP-A and SP-D levels of patients with different pulmonary diseases are presented in figures 1

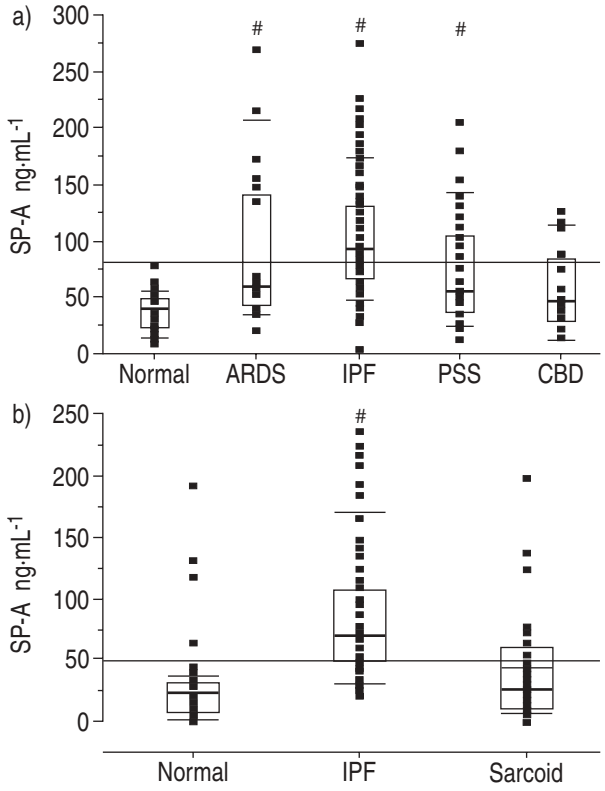

Fig. 1. – Surfactant protein-A (SP-A) concentrations in serum from a) normal volunteers (n=46), patients with acute respiratory distress syndrome (ARDS, n=21), idiopathic pulmonary fibrosis (IPF, n=142), progressive systemic sclerosis (PSS, n=37) and chronic beryllium disease (CBD, n=19) at Denver, and b) normal volunteers (n=49), patients with IPF (n=68) and sarcoidosis (n=161) at the University of Iowa. The box plots show the 10th, 25th, 75th and 90th percentiles and median. The solid horizontal line represents the 50th percentile. #: $p<0.0001$ *versus* normal subjects.

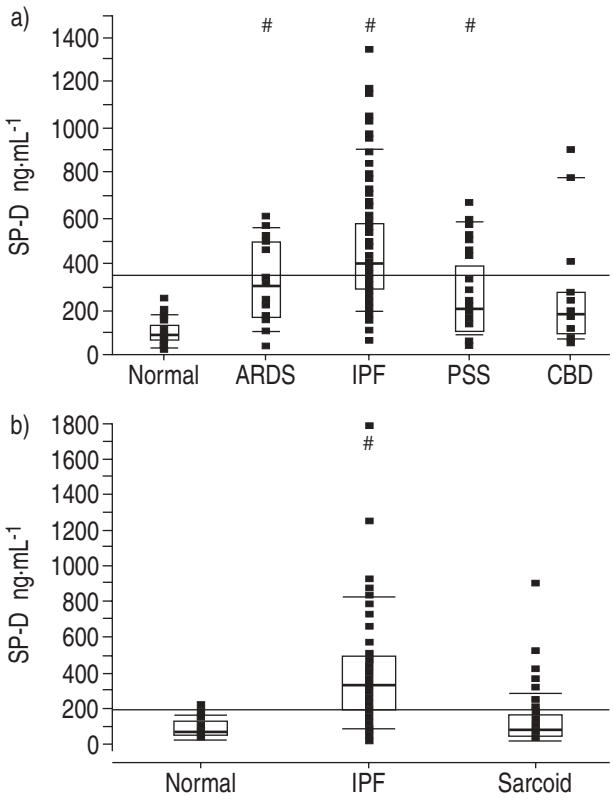

Fig. 2. – Surfactant protein-D (SP-D) concentrations in serum from a) normal volunteers (n=46), patients with acute respiratory distress syndrome (ARDS, n=21), idiopathic pulmonary fibrosis (IPF, n=142), progressive systemic sclerosis (PSS, n=37) and chronic beryllium disease (CBD, n=19) at Denver, and b) normal volunteers (n=49), patients with IPF (n=68) and sarcoidosis (n=161) at the University of Iowa. The box plots show the 10th, 25th, 75th and 90th percentiles and median. The solid horizontal line represents the 50th percentile. #: $p<0.0001$ *versus* normal subjects.

and 2. The data are organized according to site and disease category. Both SP-A and SP-D are significantly elevated in the serum of patients with IPF and PSS, but not in patients with sarcoid or beryllium disease or healthy volunteers.

Comparison of surfactant proteins-A and -D to clinical features of interstitial lung disease

To evaluate the clinical usefulness of these elevations of SP-A and SP-D, serum values were compared to pulmonary physiological variables and clinical course in each disease group. In general, there was a better correlation of serum SP-D than SP-A values with physiology and radiographical involvement.

Patients enrolled from Denver. Idiopathic pulmonary fibrosis. In the National Jewish patients, surfactant protein levels were compared to the Clinical Radiographic Physiologic (CRP) score, which includes a dyspnoea index, evaluation of a plain chest radiograph, routine pulmonary functions, and an arterial line formal exercise study [17]. Because many of the subjects did not have formal exercise testing, complete CRP scores were obtained for only 56 patients. The scores ranged from 7.97–81.22. No significant correlation between SP-A or SP-D levels and CRP score was found in these patients (Spearman Rho equal to 0.13 and 0.14, respectively). In addition, the authors found only small, insignificant correlations with any of the individual parameters of the CRP score and SP-A and SP-D measures.

In the patients with IPF from Iowa, chest radiographs were interpreted by three experienced readers, who used the ILO 1980 classification of radiographs of pneumoconiosis. Agreement between at least two of the three readers was required to identify the ILO major category of profusion. There was a significant correlation between SP-D and profusion score (Spearman coefficient=0.45; p=0.008), whereas the correlation between SP-A and profusion score was not significant (0.31; p=0.073). Hence, serum SP-D values correlated significantly with extent of disease, as determined by ILO classification in patients with IPF. The chest radiographs of the National Jewish cohort were not classified by the ILO method and could not be evaluated further.

Chronic beryllium disease. Serum SP-A and SP-D levels in patients with CBD did not differ overall

from normals. However, there were significant differences in serum SP-D levels in patients with abnormal chest radiographs compared to those with normal films (217.5 ng·mL^{-1} *versus* 82.8 ng·mL^{-1}; p=0.028). Serum SP-A levels in CBD patients with abnormal chest radiographs were not significantly different from patients with normal films (64.8 ng·mL^{-1} *versus* 34.0 ng·mL^{-1}; p=0.29).

Patients enrolled from Iowa. Sarcoid. The data from the Iowa sarcoid subjects and profusion scores were used to determine whether SP-A and SP-D levels differed for those with and without parenchymal abnormalities. An average score for profusion across the multiple B readers was used, and parenchymal abnormalities were considered present if half or more of the readers read the radiograph as having parenchymal abnormalities. A significant increase in median serum SP-D levels in patients with parenchymal abnormalities was found, compared to those without parenchymal abnormalities (92.5 ng·mL^{-1} *versus* 60.6 ng·mL^{-1}; p=0.030). The medians did not differ significantly in SP-A (p=0.125). There was a significant correlation of serum SP-D levels with mean profusion score (Rho=0.197; p=0.013), but no correlation with serum SP-A levels.

Relationship between serum surfactant proteins-A and -D levels and survival time in idiopathic pulmonary fibrosis

The authors investigated whether there was a relationship between survival time and serum SP-A or SP-D levels in the National Jewish patients with IPF. The rationale for analysing these data was to determine the independent utility of measuring serum SP-A or SP-D as a predictor of survival time in IPF. Previous studies found age and smoking status (current smokers, former smokers, and never-smokers at initial visit) to be important predictors of survival time [10]. Figure 3 shows three survival curves for the different smoking status groups in the National Jewish cohort used in this study. The survival curve for current smokers is the best, while the worst survival is seen in former smokers. Because of this and previous studies demonstrating the effects of smoking status and age on survival time in IPF, analyses evaluating the ability of serum SP-A and -D to predict survival time were adjusted for these factors.

A Cox's proportional hazards model was used to assess the relationship between serum SP-A and SP-D levels, adjusting for smoking status and age. SP-A and SP-D were analysed separately. Figure 4 shows predicted survival curves for 64-yr-old never-smokers with three different levels of SP-A; the 25th, 50th, and 75th percentiles of the observed SP-A values (68.8, 92.6, and 130.0 ng·mL^{-1}, respectively). Serum SP-A was a significant predictor of survival in this model (p<0.001). The estimated 5-yr survival percentages for a group of 64-yr-old never-smokers at the 25th, 50th, and 75th percentiles of SP-A were 48, 42 and 35%, respectively.

Similarly, the authors generated predicted survival

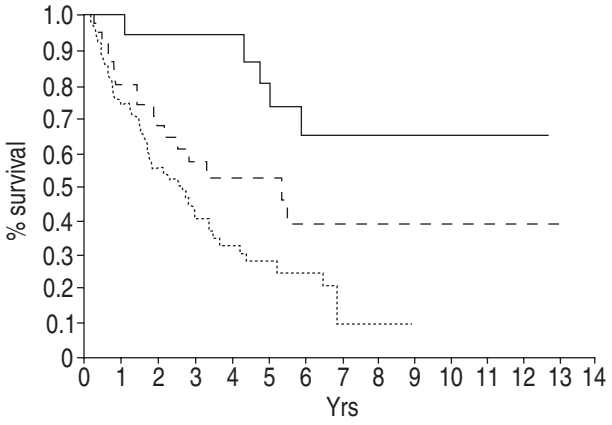

Fig. 3.–Survival analysis of patients with idiopathic pulmonary fibrosis. Kaplan-Meier survival curves by smoking status. Patients who were currently smoking (n=20; ——), never smoked (n=44; - - -) and smoked previously (n=78; ·····), had a 5-yr survival of 75.5%, 53.8% and 28.3%, respectively.

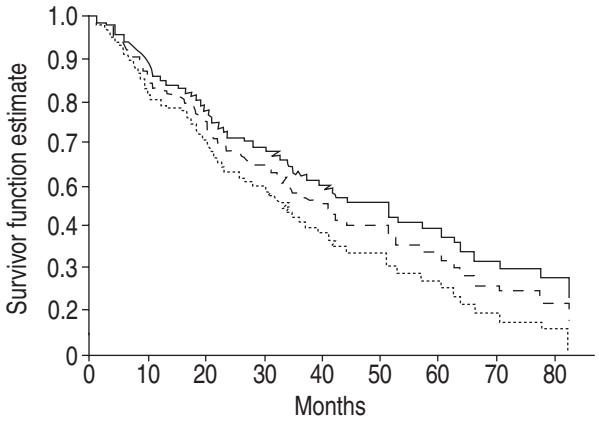

Fig. 4.–Estimated survival curves for 64-yr-old never-smoking idiopathic pulmonary fibrosis patients for three levels of surfactant protein-A (SP-A), based on Cox's proportional hazards model. ——: SP-A 69 ng·mL^{-1}; - - -: SP-A 93 ng·mL^{-1}; ·····: SP-A 130 ng·mL^{-1}.

curves for 64-yr-old never-smokers with a serum SP-D level (fig. 5) at the 25th, 50th, and 75th percentiles (290.8, 392.3, 571.4 ng·mL^{-1}, respectively). Serum SP-D was also a significant predictor of survival (p<0.001). The estimated 5-yr survival percentages for a group of 64-yr-old never-smokers at the 25th, 50th, and 75th percentiles of SP-D were 52, 45 and 35%, respectively. Although both serum SP-A and SP-D levels predict survival, serum SP-D appears to be a better predictor by Akaiuke's information criteria (statistical analysis).

Serial measurements

The initial surfactant protein level was chosen to correlate with survival because the authors examined serial surfactant protein levels in 19 National Jewish patients with IPF and found no significant changes with time (data not shown). It should be noted, however, that these patients were relatively stable

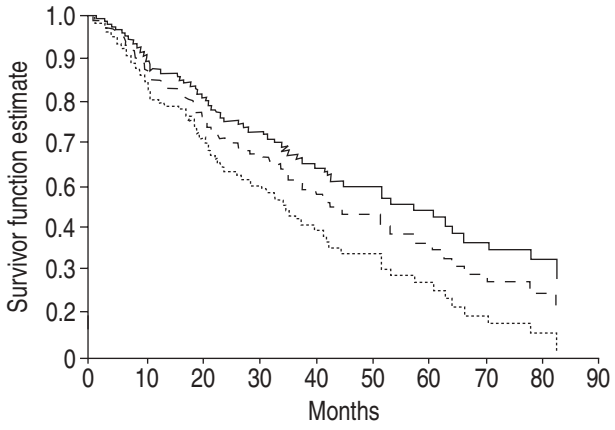

Fig. 5. – Estimated survival curves for 64-yr-old never-smoking idiopathic pulmonary fibrosis patients for three levels of surfactant protein-D (SP-D), based on Cox's proportional hazards model. ——: SP-D 291 ng·mL^{-1}; - - -: SP-D 392 ng·mL^{-1}; ·····: SP-D 571 ng·mL^{-1}.

with no large changes in the CRP score, so that the authors were unable to determine whether changes in serum SP-A or SP-D levels reflected clinical improvement or worsening.

Surfactant proteins and other commonly used predictors of survival in idiopathic pulmonary fibrosis

The study determined whether the measurement of serum SP-A or SP-D could be used to improve upon some commonly measured predictors of survival: profusion score, pulmonary hypertension, dyspnoea score, TLC % pred, forced vital capacity (FVC) % pred, forced expiratory volume in one second (FEV_1) % pred, carbon monoxide diffusion capacity ($D_{L,CO}$) % pred, and resting alveolar-arterial oxygen gradient ($P_{A\text{-}a,O_2}$). A Cox's proportional hazards model with smoking status, age and one of the commonly measured physiological parameters was fitted to the data (table 2). The results of considering one physiological parameter at a time (adjusted for age and smoking status) are summarized in table 3. The hazard's ratio of 2.04 estimated for the log of SP-D, indicates that a one-unit increase in the log of SP-D results in about twice the instantaneous risk of death due to ILD.

Table 2. – Cox's proportional hazards model

Variable	Hazard ratio	p-value	n
ln (SP-A)	1.73	0.031	142
ln (SP-D)	2.04	0.003	142
Profusion	1.63	<0.001	141
Pulmonary hypertension	2.36	0.002	141
Dyspnoea	1.05	0.054	142
TLC % pred$^{\#\P}$	1.52	0.039	142
FVC % pred$^{\#\P}$	1.31	0.031	142
FEV_1 % pred$^{\#\P}$	1.22	0.009	142
$D_{L,CO}$ % pred$^{\#\P}$	1.36	0.030	138
Resting $P_{A\text{-}a,O_2}{}^{\#+}$	1.34	0.036	137

SP-A: surfactant protein-A; SP-D: surfactant protein-D; TLC: total lung capacity; FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; $D_{L,CO}$: carbon monoxide diffusion capacity; $P_{A\text{-}a,O_2}$: alveolar-arterial oxygen gradient; % pred: % predicted. $^{\#}$: indicates that hazards were not proportional, and the hazard ratio listed is the 1 yr estimate, while the p-value is the p-value testing for proportional hazards; ¶: indicates that the hazard ratio is for a 10 unit decrease; $^{+}$: indicates that the hazard ratio is for a 10 unit increase.

Table 3. – Serum surfactant proteins-A (SP-A) and -D (SP-D) levels in healthy volunteers according to site study

	Denver	Iowa	Japan
SP-A ng·mL^{-1}			
n	46	49	246
Median	39.60	22.20	21.20
75th percentile	49.40	31.55	29.00
25th percentile	24.28	10.10	18.00
SP-D ng·mL^{-1}			
n	46	49	131
Median	97.25	86.98	42.67
75th percentile	127.86	125.33	60.67
25th percentile	71.31	61.67	33.36

In addition to looking at singular physiological parameters, the authors ran a multivariate model to determine the additional predictive value of SP-D after accounting for age, smoking status, TLC % pred, radiograph profusion score, FVC % pred, FEV_1 % pred, $D_{L,CO}$ % pred, and the resting $P_{A\text{-}a,O_2}$ difference. For both TLC and the $P_{A\text{-}a,O_2}$ difference, the assumptions of proportional hazards were not met and thus interactions of time with each of these variables were maintained in the multivariate model. In the multivariate model, the log of the serum SP-D level was only of borderline significance (hazard ratio=1.559; p=0.083). Hence, serum SP-D and SP-A were independent predictors of survival time in patients with IPF. The multivariate analysis suggests that serum SP-A and SP-D levels do not appear to improve upon commonly measured predictors of survival in patients with IPF.

Discussion

Type II cells are the cuboidal, alveolar epithelial cells responsible for producing and secreting surfactant and most of the surfactant-associated proteins (SP-A, SP-B, SP-C and SP-D) into the alveolar lining fluid [18]. SP-A and SP-D are hydrophilic glycoproteins that are structurally similar to the circulating proteins, mannose-binding protein and bovine conglutinin, respectively [19–21], whereas SP-B and SP-C are very hydrophobic proteins [19, 22], which would not be expected to be found in circulation. The concentration of the surfactant proteins in serum and alveolar lining fluid varies in disease. There is an increase in surfactant proteins in BAL fluid from patients with alveolar proteinosis [6] and a decrease in BAL surfactant proteins in acute respiratory distress syndrome (ARDS), IPF and pneumonia [5, 16, 23–26]. McCormack *et al.* [23] also reported a decrease in the ratio of SP-A/phospholipid (to correct for total surfactant recovery) in lavage fluid

from patients with IPF. The SP-A/phospholipid ratio predicted 5-yr survival better than any other lavage constituent measured, which included the cell count and differential [23]. Interestingly, lavage SP-D levels in patients with IPF do not increase [16, 23]. Serum SP-A [6] and SP-D [16] levels have also been studied in a small number of patients with IPF and both show an increase when compared to controls. In addition, TAKAHASHI *et al.* [27] found significantly higher serum SP-A and SP-D levels in 10 patients with IPF who died, compared to 42 who were still alive at the 3-yr follow-up period.

The objective of this study was to determine whether serum SP-A or SP-D levels would be elevated in patients with IPF compared to patients with other ILDs, and whether these levels would be clinically useful in predicting survival time in a large number of patients when compared to other commonly used predictors of survival. The authors found that SP-A and SP-D are elevated in serum from patients with IPF and PSS, but not in patients with sarcoid or beryllium disease. If patients with sarcoid or beryllium disease with significant parenchymal disease were observed however, there was a correlation with serum SP-D levels.

What causes elevated surfactant proteins-A and -D in serum?

There are several possible mechanisms for producing an elevation of SP-A or SP-D in serum. These include increased secretion of SP-A or SP-D per type II cell, an increase in total type II cells per lung due to diffuse hyperplasia, increased leak from the airspace to the interstitium, and decreased clearance from the vascular compartment. It is probable that epithelial injury and leak contributes significantly to serum levels of SP-A and SP-D and that the levels do not simply reflect the mass of type II cells within the lung. However, this paper does not address the mechanism(s) responsible for the increases in serum surfactant proteins.

Smoking status and survival

One of the more interesting findings of this study was the correlation of smoking with survival in patients with IPF. The reason why current smokers have a better outcome than patients who have never smoked is not clear. The smokers may have had underlying obstructive disease, which might have produced symptoms earlier so that their disease was recognized earlier. However, the hazard curves are not parallel, which suggests that the time of presentation of the disease is not the critical determinate. It is known that smoking protects against hypersensitivity pneumonitis and worsens eosinophilic granuloma and respiratory bronchiolitis associated with ILD.

Therefore, there may be some basic element in the pathophysiology of IPF that is altered by cigarette smoking. An alternative explanation is that the airspace enlargement and alveolar septal wall disruption, *i.e.* emphysema, produced by cigarette smoking prevents apositional atelectasis and fusion of adjacent alveolar walls, which is thought to be critical in the formation of pulmonary fibrosis and loss of alveolar units [28]. Nevertheless, the former smokers had a worse prognosis. Another possible explanation is that smoking may alter the pathogenesis of IPF, as the effect appears to be related to current smoking.

The diagnosis and management of IPF poses a significant challenge for the clinician. Although the disease course often spans >10 yrs, many of the patients present late in the illness. Current markers of clinical outcome are predominantly based on abnormal physiological and radiographical findings, all of which occur later in the disease process. To date, there are no good markers that predict survival time early in the course of the disease. The authors have reported previously that SP-A is reduced in lavage of patients with IPF, and that SP-A levels predict survival in this patient population [23]. However, because of the difficulty in obtaining frequent lavage fluid samples from these patients, the authors wanted to determine whether serum measurements of SP-A or SP-D might be justified in the clinical setting. Serum SP-A and SP-D have already been measured as biomarkers in patients with other pulmonary diseases, including ARDS [25] and progressive systemic sclerosis [9], and some chest physicians feel that the measurement of these proteins in serum is helpful to differentiate some interstitial pneumonias from other types of ILDs. In the authors′ experience however, this is not a commonly held view. In this study, it was demonstrated that while both serum SP-A and SP-D levels predict survival, SP-D appears to be better related to parenchymal involvement. In addition, in a large number of patients, it was found that serum SP-A and SP-D are highly predictive of survival in IPF.

The final issue that needs further investigation is the usefulness of changes in serum levels of surfactant protein-A or -D during the clinical course of patients with diffuse lung disease. It seems logical that serum levels would change during the course and reflect disease activity. This occurred in a small cohort of patients with idiopathic pulmonary fibrosis in a previous study [27] and occurs in bleomycin injury in rats [29]. However, in the few patients that had serial measurements in this study, there was little change in the absolute values and in the Clinical Radiographic Physiologic score. Hence, the authors were not able to determine whether serum values change with disease activity. Future studies with more clinically-responsive interstitial lung diseases, such as bronchiolitis obliterans with organizing pneumonia and nonspecific interstitial pneumonitis, will be needed to determine whether the serum levels vary with disease activity. Since the serum levels of surfactant protein-D appear to be related to the extent of parenchymal disease, the authors are hopeful that they may be useful in these more responsive forms of interstitial lung disease.

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