Serum surfactant protein D as a prognostic factor in idiopathic pulmonary fibrosis

El-Miligy Dawalat[a], Zakaria Mohamed W.[b], Rashed Laila[a], Abu-Hussein Haidi[c]

Background/aim The aim of this study was to investigate the possible role of surfactant protein D (SP-D) in the pathogenesis and the prognosis of idiopathic pulmonary fibrosis (IPF).

Materials and methods This study was performed on 10 healthy volunteers (group 1) and 30 patients from the Chest Diseases Department, Kasr El-Aini Hospital, who were divided into three groups: group 2 consisted of 10 patients with IPF receiving steroids; group 3 consisted of 10 patients with IPF not receiving steroids; and group 4 consisted of 10 patients with chronic chest diseases other than IPF and not receiving steroids. All patients underwent full history taking, thorough clinical examination, chest radiography and high-resolution computed tomography of the chest, pulmonary function testing, and estimation of SP-D by enzyme-linked immunosorbent assay.

Results There was no statistical significance in the mean age of the four included groups. With regard to smoking in patients in groups 2, 3, and 4, there was no statistical significance in the duration or the number of cigarettes smoked per day. There was a significant decrease in FEV_1, FVC, and FEV_1/FVC in groups 2, 3, and 4 compared with the control group (group 1). SP-D shows a significant increase in groups 2, 3, and 4 compared with the control group, and also shows a significant increase in IPF patients not receiving steroids (group 3) compared with IPF patients receiving steroids (group 2) and patients with chronic chest diseases (group 4). A negative correlation was found between SP-D and FEV_1, FVC, and FEV_1/FVC. No correlation was found between SP-D, age, the duration of smoking, or the number of cigarettes smoked per day.

Conclusion The SP-D assay may indicate the rate of decline in the pulmonary function in cases of IPF and in the follow-up of disease progress. It may also assist in making clinical choices for the therapeutic management of patients with IPF. *Egypt J Broncho* 2015 9:64–68
© 2015 Egyptian Journal of Bronchology.

Egyptian Journal of Bronchology 2015 9:64–68

Keywords: idiopathic pulmonary fibrosis, prognostic factor, surfactant protein D

[a]Departments of Biochemistry, [b]Chest Diseases, Cairo University, Cairo and [c]Department of Biochemistry, 6th October University, Egypt

Correspondence to Mohamed W. Zakaria, Department of Chest Diseases, Cairo University, 5, Makrize Street, Zamalek, Cairo 11211, Egypt
e-mail: mw_khalil@hotmail.com

Received 10 April 2014 **Accepted** 18 April 2014

Introduction

Idiopathic pulmonary fibrosis (IPF) is defined as a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause, occurring primarily in older adults, limited to the lungs, and associated with the histopathologic and/or radiologic pattern of UIP [1].

IPF is a progressive, life-threatening, interstitial lung disease of unknown etiology. For optimal therapeutic management of IPF, an accurate tool is required for discrimination between reversible and irreversible types of the disease. However, such noninvasive tools are few, and even with high-resolution computed tomography, which is the most trusted method for this purpose, the nature of the disease activity in IPF cannot always be predicted accurately [1].

Surfactant protein 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 [2]. Although the exact mechanism of the increase in 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-D probably vary with disease and lung inflammation, measurement of these two proteins might prove to be useful markers for the pathogenesis and detection of IPF [3–5].

This study aims to investigate the possible role of SP-D in the pathogenesis and the prognosis of IPF.

Materials and methods

The present work was conducted on 10 healthy volunteers and 30 patients from the Department of Chest Diseases, Faculty of Medicine, Cairo University, during the period from November 2009 to May 2010 and they were categorized into four groups as follows:

(1) *Group 1*: Ten healthy volunteers (control group, six male and four female): nonsmokers aged 54.7 ± 7.13 years (mean ± SD) (range: 45–65 years).
(2) *Group 2*: Ten patients with IPF receiving steroid therapy for 1 month (six male and four female), consisting of two current smokers, four ex-smokers, and four nonsmokers aged 57 ± 6.63 years (mean ± SD) (range: 45–65 years).

(3) *Group 3*: Ten patients with IPF not receiving steroid therapy (five male and five female), consisting of two current smokers, three ex-smokers, and five nonsmokers aged 54.3 ± 5.27 years (mean ± SD) (range: 45–65 years).
(4) *Group 4*: Ten patients with chronic chest disease without IPF and not receiving steroids (five male and five female), consisting of two current smokers, three ex-smokers, and five nonsmokers aged 55.1 ± 8.17 years (mean ± SD) (range: 45–65 years).

Inclusion criteria

All IPF patients fulfilled the major and minor criteria of ATS/ERS consensus classification of the idiopathic interstitial pneumonias.

Exclusion criteria

(1) All known causes of ILD: for example, connective tissue diseases, sarcoidosis, or drug induced.
(2) Malignancy and other cause of interstitial pneumonia.

Patients were subjected to the following:
(1) Full history taking.
(2) Full clinical examination.
(3) Chest radiography and high-resolution computed tomography of the chest.
(4) Pulmonary function tests.
(5) Determination of serum SP-D levels.

Collection and analysis of blood samples

Ten milliliters peripheral venous blood samples were collected from the patients at their initial visits and from healthy participants at the time of registration for the study. The serum samples were stored at -80°C and analyzed in a blinded manner with regard to the clinical status of the patients.

Determination of surfactant protein D

The SP-D assay was performed with enzyme-linked immunosorbent assay kits provided by the Teijin Institute of Bio-medicine (Tokyo, Japan). A method based on that of Shimizu *et al.* [6] was adapted with minor modifications [7]. The concentration of SP-D was measured using recombinant SP-D as a standard and two monoclonal antibodies against human SP-D [8]. This assay system was able to detect SP-D at concentrations of 1.56–100 ng/ml. All assays were performed in duplicate, and the results are given as the mean value. In immunoblot analyses, specific antibodies used for the SP-D assay systems showed no nonspecific cross-reactivity, such as with mannose-binding protein or with other serum proteins.

Statistical analysis

Data are expressed as mean ± SD. Differences between SP-D values in the three study groups, variables were assessed with the Mann–Whitney *U*-test. Concentrations of SP-D were further analyzed using the Student *t*-test for the healthy group to find the cutoff levels indicating the best sensitivity and specificity of these two measures [9]. Significance was defined as *P*-value less than 0.001.

Results

In our study, the mean age of the control group was 54.70 ± 7.13 years and the mean age of the steroidal group was 57.00 ± 6.63 years.

The mean age of the nonsteroidal group was 54.30 ± 5.27 years, whereas that of the chronic group was 55.10 ± 8.17 years. There was no significant difference between the four groups.

Among current smokers, the mean duration of smoking per month was 25.00 ± 7.07 months among chronic patients, whereas 21.50 ± 4.95 months among nonsteroidal current smokers and 15.00 ± 7.07 months among steroidal current smokers; no significant differences was detected between the three groups.

In addition, the mean number of cigarettes smoked per day by current smokers was 120.00 ± 33.94 cigarettes among chronic patients, whereas it was 75.00 ± 21.21 cigarettes among nonsteroidal current smokers and 126.00 ± 8.49 cigarettes among steroidal current smokers; there was no significant difference between the three groups.

Table 1 shows a statistically significant decrease in FEV_1, FVC, and FEV_1/FVC (%) in the three disease groups compared with the control group. No significant difference was detected between the three disease groups.

The following observations can be made from Table 2.

Table 1 Mean ± SD and *P*-value of the pulmonary function test in the study groups

PFT	Control group	Steroidal group	Nonsteroidal	Chronic group
FEV_1 (l)	3.13 ± 0.72	1.98 ± 0.49	1.86 ± 0.64	1.71 ± 0.44
FVC (l)	3.11 ± 0.69	2.01 ± 0.49	1.88 ± 0.65	1.71 ± 0.44
FEV_1/FVC (%)	3.16 ± 0.72	2.05 ± 0.56	1.91 ± 0.58	1.58 ± 0.43
P-values	P_1 = 0.001	P_1 = 0.001	P_2 = 0.000	P_3 = 0.000
	P_2 = 0.000	P_4 = 1.000	P_4 = 1.000	P_5 = 0.474
	P_3 = 0.000	P_5 = 0.474	P_6 = 1.000	P_6 = 1.000

Values are represented as mean ± SD; PFT, pulmonary function test; P_1 = significant difference between the control group and the steroidal group; P_2 = significant difference between the control group and the nonsteroidal group; P_3 = significant difference between the control group and the chronic group; P_4 = significant difference between the steroidal group and the nonsteroidal group; P_5 = significant difference between the steroidal group and the chronic group; P_6 = significant difference between the nonsteroidal group and the chronic group; *Values are significant when $P < 0.05$.

Table 2 Mean ± SD and *P*-value of the serum surfactant protein D (ng/ml) level in the different study groups

	Control group	Steroidal group	Nonsteroidal	Chronic group
SP-D (ng/ml)	50.12 ± 12.17	136.06 ± 38.15	253.18 ± 37.63	198.50 ± 38.50
P-values	P_1 = 0.000	P_1 = 0.000	P_2 = 0.000	P_3 = 0.000
	P_2 = 0.000	P_4 = 0.000	P_4 = 0.000	P_5 = 0.001
	P_3 = 0.000	P_5 = 0.001	P_6 = 0.005	P_6 = 0.005

Values are represented as mean ± SD; SP-D, surfactant protein D; P_1 = significant difference between the control group and the steroidal group; P_2 = significant difference between the control group and the nonsteroidal group; P_3 = significant difference between the control group and the chronic group; P_4 = significant difference between the steroidal group and the nonsteroidal group; P_5 = significant difference between the steroidal group and the chronic group; P_6 = significant difference between the nonsteroidal group and the chronic group; *Values are significant when $P < 0.05$.

(1) There is a significant increase in SP-D in the three disease groups compared with the control group.
(2) There is a significant increase in SP-D in the nonsteroidal and the chronic groups compared with the steroidal group; there is also a significant increase in the nonsteroidal group compared with the chronic group (the nonsteroidal group showed the highest mean values).

The following observations can be made from Table 3.

A negative correlation was found between the SP-D serum level and FEV_1 (l), with *P*-value greater than 0.000 and *r*-value of -0.541.

In addition, a negative correlation was found between the SP-D serum level and FVC (l), with *P*-value greater than 0.000 and *r*-value of -0.530.

Also, a negative correlation was found between the SP-D serum level and FEV_1/FVC, with *P*-value greater than 0.000 and *r*-value of -0.546.

In Table 4, no correlation was found between the SP-D serum level and the duration of smoking per month, with *P*-value less than 0.146 and *r*-value of -0.669.

In addition, no correlation was found between the SP-D serum level and the number of cigarettes smoked per day, with *P*-value less than 0.433 and *r*-value of 0.399.

Table 5 shows no correlation between the SP-D serum level and the age, with *P*-value less than 0.678 and *r*-value of 0.068 (Figs 1–4).

Table 3 The serum surfactant protein D level (ng/ml) in relation to pulmonary function tests

Pulmonary function tests	Correlation	SP-D in serum
FEV_1 (l)	*r*-value	-0.541
	P-value	0.000
FVC (l)	*r*-value	-0.530
	P-value	0.000
FEV_1/FVC (%)	*r*-value	-0.546
	P-value	0.000

SP-D, surfactant protein D.

Table 4 The serum surfactant protein D level (ng/ml) in relation to the smoking duration per month and the number of cigarettes per day

Smoking status	Correlation	SP-D in serum
Duration of smoking per month	*r*-value	-0.669
	P-value	0.146
Number of cigarettes per day	*r*-value	0.399
	P-value	0.433

SP-D, surfactant protein D.

Table 5 The serum surfactant protein D level (ng/ml) in relation to age

	Correlation	SP-D in serum
Age	*r*-value	0.068
	P-value	0.678

SP-D, surfactant protein D.

The results of the study showed that pulmonary function tests were significantly decreased in the three disease groups compared with the control group. No significant difference was detected between the three disease groups. Regarding the duration of smoking and the number of cigarettes smoked/day, no significant difference was detected between the three patient groups.

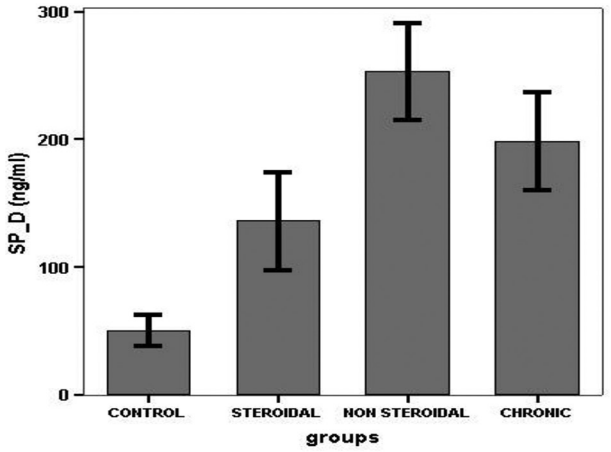
Collard *et al*. [11] demonstrated a strong association between cigarette smoking and pulmonary fibrosis.

The results of SP-D in the present study showed a significant increase in the three disease groups compared with the control group. Moreover, the value was significantly higher in the nonsteroid and the chronic groups compared with the steroid-receiving group. Also, the value was significantly higher in the nonsteroid group than in the chronic group.

Discussion

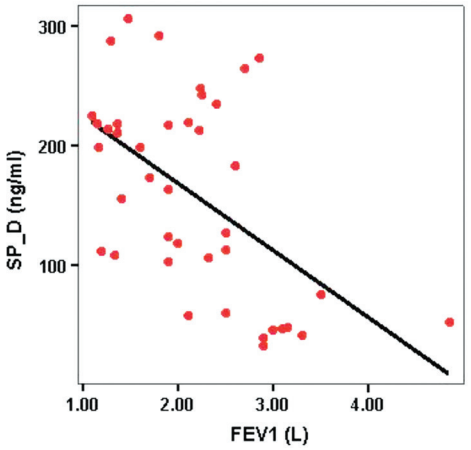
Regarding the relation between the age of the patients and the development of IPF, many reports proved that the incidence undoubtedly increases with age. All patients enrolled in our study were above 50 years of age. This is in agreement with the study of Thomas *et al*. [10], who mentioned that patients with IPF are usually between 40 and 70 years of age. Also, the authors found that two-third of the IPF cases present over the age of 60 years at the time of diagnosis.

Fig. 1



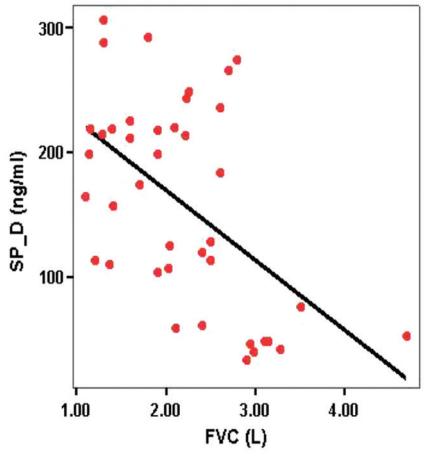
Mean ± SD of serum surfactant protein D level (ng/ml) in the different study groups.

Fig. 2



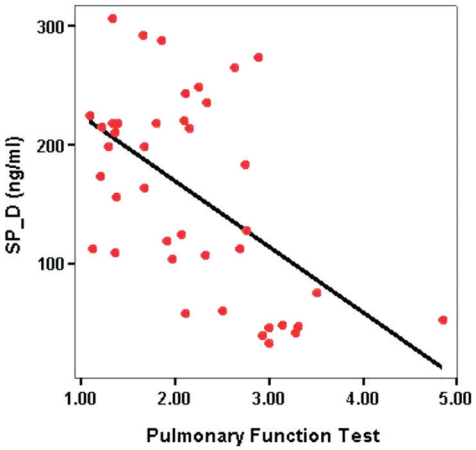
The serum surfactant protein D level (ng/ml) in relation to FEV_1 (l).

Fig. 3



The serum surfactant protein D level (ng/ml) in relation to FVC (l).

Fig. 4



The serum surfactant protein D level (ng/ml) in relation to pulmonary function tests.

Schwartz *et al.* [12] stated that patients exhibiting higher serum levels of SP-D may have a greater chance of developing restrictive pulmonary dysfunction, and more rapidly than patients with low serum levels of SP-D. He also concluded that it may be more effective to start treatment for IPF before the manifestations of severe pulmonary fibrosis occur. Our results raise the possibility that the assay of SP-D can help guide the therapy with corticosteroids agents.

McCormack *et al.* [13] evaluated the utility of assays of serum SP-D in establishing the prognosis of patients with IPF. None of the patients showing SP-D levels below the respective levels died throughout the period of the study (2 years). His findings suggest that an SP-D assay is useful to identify patients with the best prognosis in IPF.

A difference between levels of SP-D was also observed in the four studied groups. Honda *et al.* [14] concluded that the concentration of serum SP-D varies with disease severity and lung inflammation and that differences in response to corticosteroid products and solubility could also affect concentrations of SP-D in the serum.

This finding agrees with our study, where we found the level of SP-D to be lower in IPF patients receiving corticosteroid compared with those not receiving corticosteroids and the group with chronic disease.

Takahashi [15] found that the concentrations of SP-A and SP-D in patients who died within 3 years were significantly higher than in patients who were still alive after 3 years. It has been proposed that assays of SP-A and SP-D in sera from IPF patients are useful tools for understanding some pathologic characteristics of the disease, that SP-D may be a good predictive indicator of the rate of decline in pulmonary function, and that a combination of the assays for SP-A and SP-D may be

helpful in predicting the outcome of patients with IPF.

This study is in agreement with our results where a negative correlation was found between the SP-D level and FEV_1, FVC, and FEV_1/FVC.

Part of our results is in agreement with data from Takahashi *et al*. [16] and Barlo *et al*. [17] and it showed that SP-D in the serum can predict worsening in IPF patients, and that the value of SP-D remains stable after adjustment for known predictors of worsening. The author mentioned that a serum SP-D level higher than 460 ng/ml indicates a significantly worse prognosis compared with levels lower than 460 ng/ml. This value can be useful in clinical practice. It might help in estimating the survival time, which is important for the optimal timing of referral for lung transplantation [16].

According to Kinder *et al*. [18], an increased serum SP-D level is a strong and independent predictor of early mortality among patients with IPF. A prediction model containing SP-A and SP-D was substantially superior to a model with clinical predictors alone.

SP-D is a noninvasive marker that can be easily determined in the serum and has been proven to be a diagnostic marker in IPF patients. This study adds clinically useful levels that could identify patients with a significantly worse prognosis using SP-D for the follow-up of the patients. This prognostic value of SP-D persists after adjustment for known predictors of mortality.

Acknowledgements

Conflicts of interest

None declared.

References

1 American Thoracic Society (ATS)/European Respiratory Society (ERS). American Thoracic Society (ATS)/European Respiratory Society (ERS) International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am J Respir Crit Care Med* 2002; **165**:277–304.

2 Jeffrey AW, Ann DH. 2008. *Fishman's pulmonary diseases and disorders*. Vol. 1 and 2, ISBN: 0-07-145739-9 4th ed.

3 Ikegami M, Clinton NA, Korfhagen TR, Whitsett JA. Surfactant protein D influences surfactant ultrastructure and uptake by alveolar type II cells. *Am J Physiol* 2005; **288**:L552.

4 Wert SE, Yoshida M, LeVine AM, Ikegami M, Jones T, Ross GF, *et al*. Increased metalloproteinase activity, oxidant production, and emphysema in surfactant protein D gene-inactivated mice. *Proc Natl Acad Sci USA* 2000; **97**:5972.

5 Whitsett JA, Weaver TE. Hydrophobic surfactant proteins in lung function and disease. *N Engl J Med* 2002; **347**:2141.

6 Shimizu H, Hosoda K, Mizumoto M, Kuroki Y, Sato H, Kataoka K, *et al*. Improved immunoassay for the determination of surfactant protein A (SP-A). *Tohoku J Exp Med* 1989; **157**:269–278.

7 Honda Y, Kuroki Y, Matsuura E, Nagae H, Takahashi H, Akino T, Abe S. Pulmonary surfactant protein D in sera and bronchoalveolar lavage fluids. *Am J Respir Crit Care Med* 1995a; **152**:1860–1866.

8 Nagae H, Takahashi H, Kuroki Y, Honda Y, Nagata A, Ogasawara Y, *et al*. Enzyme-linked immunosorbent assay using F(ab')2 fragment for the detection of human pulmonary surfactant D in sera. *Clin Chim Acta* 1997; **266**:157–171.

9 Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plot: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993; **39**: 561–577.

10 Thomas AQ, Lane K, Phillips JIII. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. *Am J Respir Crit Care Med* 2002; **165**:1322.

11 Collard HR, King TE, Bartelson BB, Vourlekis JS, Schwarz MI, Brown KK. Changes in clinical and physiological variables predict survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2003; **168**:538–542.

12 Schwartz DA, VanFossen DS, Davis CS, Helmers RA, Dayton CS, Burmeister LF, Hunninghake GW. Determinants of progression in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2005; **149**:444–449.

13 McCormack FX, King TE, Voelker DR, Robinson PC, Mason RJ. Idiopathic pulmonary fibrosis: abnormalities in the bronchoalveolar lavage content of surfactant protein A. *Am Rev Respir Dis* 1991; **144**:160–166.

14 Honda Y, Kuroki Y, Shijubo N, Fujishima T, Takahashi H, Hosoda K, *et al*. Aberrant appearance of lung surfactant protein A in sera of patients with idiopathic pulmonary fibrosis and its clinical significance. *Respiration* 1995b; **62**:64–69.

15 Takahashi H. *Am J Respir Crit Care Med* 2000; **162**:1109–1114.

16 Takahashi H, Fujishima T, Koba H. Serum surfactant proteins A and D as prognostic factors in idiopathic pulmonary fibrosis and their relationship to disease extent. *Am J Respir Crit Care Med* 2000; **162**:1109–1114.

17 Barlo NP, van Moorsel CH, Ruven HJ, Zanen P, van-den Bosch JM, Grutters JC. Surfactant protein-D predicts survival in patients with idiopathic pulmonary fibrosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2009; **26**:155–161.

18 Kinder BW, Brown KK, McCormack FX, Ix JH, Kervitsky A, Schwarz MI, King TE. Serum surfactant protein-A is a strong predictor of early mortality in idiopathic pulmonary fibrosis. *Chest* 2009; **135**:1557–1563.