Serum surfactant protein D as a prognostic factor in idiopathic pulmonary fibrosis
El-Miligy Dawalata, 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 FEV1, FVC, and FEV1/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 FEV1, FVC, and FEV1/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.

Keywords: idiopathic pulmonary fibrosis, prognostic factor, surfactant protein D
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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 based on that of Shimizu et al. [6]. This assay system was able to detect SP-D and two monoclonal antibodies against human surfactant protein D [8]. This assay system was able to detect SP-D and two monoclonal antibodies against human surfactant protein D [8].

**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 FEV1, FVC, and FEV1/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

<table>
<thead>
<tr>
<th>PFT</th>
<th>Control group</th>
<th>Steroidal group</th>
<th>Nonsteroidal group</th>
<th>Chronic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1 (l)</td>
<td>3.13 ± 0.72</td>
<td>1.98 ± 0.49</td>
<td>1.86 ± 0.64</td>
<td>1.71 ± 0.44</td>
</tr>
<tr>
<td>FVC (l)</td>
<td>3.11 ± 0.69</td>
<td>2.01 ± 0.49</td>
<td>1.88 ± 0.65</td>
<td>1.71 ± 0.44</td>
</tr>
<tr>
<td>FEV1/FVC (%)</td>
<td>3.16 ± 0.72</td>
<td>2.05 ± 0.56</td>
<td>1.91 ± 0.58</td>
<td>1.58 ± 0.43</td>
</tr>
<tr>
<td>P-values</td>
<td></td>
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</tr>
<tr>
<td>P1 = 0.001</td>
<td>P2 = 0.001</td>
<td>P3 = 0.000</td>
<td>P4 = 0.000</td>
<td>P5 = 0.474</td>
</tr>
<tr>
<td>P1 = 0.000</td>
<td>P2 = 1.000</td>
<td>P3 = 1.000</td>
<td>P4 = 1.000</td>
<td>P5 = 1.000</td>
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</tr>
</tbody>
</table>

Values are represented as mean ± SD; PFT, pulmonary function test; P1 = significant difference between the control group and the steroidal group; P2 = significant difference between the control group and the nonsteroidal group; P3 = significant difference between the control group and the chronic group; P4 = significant difference between the steroidal group and the nonsteroidal group; P5 = significant difference between the steroidal group and the chronic group; *Values are significant when P < 0.05.
There is a significant increase in SP-D in the three disease groups compared with the control group.

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 FEV₁ (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 FEV₁/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).

**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-thirds of the IPF cases present over the age of 60 years at the time of diagnosis.

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

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.
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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 corticosteroids 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

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.

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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 FEV₁, FVC, and FEV₁/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