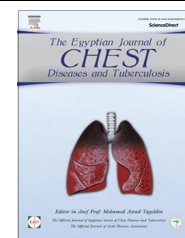HOSTED BY

The Egyptian Society of Chest Diseases and Tuberculosis

Egyptian Journal of Chest Diseases and Tuberculosis

www.elsevier.com/locate/ejcdt
www.sciencedirect.com

ELSEVIER

The Egyptian Journal of CHEST Diseases and Tuberculosis

ORIGINAL ARTICLE

Clinical significance of serum surfactant protein D in patients with rheumatoid arthritis-associated interstitial lung diseases

CrossMark

Ashraf E. Sileem [a], **Ahmed Mohamed Said** [a,*], **Ahmed Mohamed Alsowey** [b], **Sameh A. Soliman** [c]

[a] *Chest Depatment, Zagazig University, Egypt*
[b] *Radiodiagnosis Department, Zagazig University, Egypt*
[c] *Internal Medicine Department, Zagazig University, Egypt*

Abstract   *Background:* Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects approximately 1% of the population, and pulmonary involvement is common. The most common pulmonary manifestation is interstitial lung disease (ILD) which leads to pulmonary fibrosis. ILD is the only complication of RA reported to be increasing in prevalence and it has been shown to account for around 6% of all RA deaths. Surfactant proteins (SP-A, SP-B, SP-C and SP-D) play various important roles in the lung. Serum SP-D levels reflected the disease activity of pulmonary fibrosis.

Aim of the study: To determine the clinical significance of surfactant protein D (SP-D), a useful marker for evaluating interstitial lung diseases and specifically in rheumatoid arthritis associated interstitial lung disease patients.

Patients and methods: Our patients were classified into 3 groups (Group 1: 18 patients had rheumatoid arthritis ILD; Group 2: 12 patients had rheumatoid arthritis without interstitial pulmonary disease; Group 3: 10 patients had idiopathic pulmonary fibrosis with no rheumatoid arthritis) and 20 healthy control subjects. Serum SP-D levels were assayed using a specific enzyme-linked immunosorbent assay for all studied groups.

Results: A highly significant difference was found between patients' groups and the control group regarding disease duration, serum aCCP, SP-D, serum CRP and RF being higher in patients' group ($p < 0.01$). However FVC was significantly lower in patients' groups compared to the control group ($p < 0.05$). Assessment of the diagnostic performance of SP-D assay revealed that the best cutoff for discriminating rheumatoid patients with interstitial lung disease from those without interstitial lung disease was 219 ng/mL. At this value, SP-D had a diagnostic sensitivity of 94.2%, specificity 90%, negative predictive value 90%, positive predictive value 94.2% and efficiency 95%.

Conclusion: The serum SP-D level may be a useful marker for ILD especially in patients with rheumatoid arthritis.

* Corresponding author. Tel.: +20 01227308462.
E-mail address: kingarmstrong100@yahoo.com (A.M. Said).
Peer review under responsibility of The Egyptian Society of Chest Diseases and Tuberculosis.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects approximately 1% of the population, and pulmonary involvement is common. Furthermore, the lifespan of RA patients is shortened by approximately 10 years, and standardized mortality ratios for RA range from 1.28 to 3.0 [1]. Respiratory causes are a significant contributor to excess mortality in patients with RA ranking as the second major cause of death in this patient population. While the treatment of rheumatoid articular disease has greatly improved in recent years, as measured by disease activity and quality of life instruments, these benefits have not extended to RA-associated lung diseases [2].

A number of pulmonary manifestations are associated with RA. The most common is interstitial lung disease (ILD) which leads to pulmonary fibrosis (PF) during which the lung parenchyma is involved. ILD is not only the most common but also the most serious form of lung involvement in RA. Radiographic changes such as fibrosis, and physiological changes such as restriction or decreased diffusing capacity on pulmonary function testing, may precede symptoms by years; however, once clinically apparent, ILD is associated with significant mortality. Although it is common for ILD to be diagnosed concurrently with or after RA, a population based study suggests that 3.5% of patients with RA were given a diagnosis of ILD prior to the diagnosis of RA. Early studies identified a high post-mortem incidence of RA–ILD and this was subsequently supported by high-resolution computed tomography (HRCT) which confirmed that up to 25% of RA patients had ILD. ILD is the only complication of RA reported to be increasing in prevalence and it has been shown to account for around 6% of all RA deaths [3].

Pulmonary surfactant is composed of phospholipids and associated proteins. It covers the alveolar surface and maintains alveolar gas exchange during expiration. There are four distinct proteins in pulmonary surfactant, which are designated surfactant proteins: SP-A, SP-B, SP-C and SP-D. These surfactant proteins play various important roles in the lung, such as maintaining the biophysical activity of the surfactant and regulating surfactant homeostasis in the alveoli [4].

SP-D is a hydrophilic glycoprotein with a reduced molecular mass of 43 kDa. SP-D is produced and secreted by alveolar type II epithelial cells and club cells structure which gives it the ability to agglutinate pathogens, as well as aid in the clearance of apoptotic cells, cellular debris, and foreign particles in the lung. Recently, the SP-D levels in bronchoalveolar lavage (BAL) fluids and the systemic circulation have been measured in several lung diseases, including idiopathic pulmonary fibrosis. It was reported that the SP-D level in BAL fluids was decreased in patients with pulmonary fibrosis, while the serum SP-D level was significantly elevated [5].

This may be because the increased permeability of SP-D results in an increase in alveolar to- vascular leakage of SP-D in these patients. Increased serum SP-D levels were detected in patients with idiopathic pulmonary fibrosis, interstitial pneumonia with collagen diseases and pulmonary alveolar proteinosis. In contrast, the serum SP-D levels in patients with bacterial pneumonia were not elevated. Moreover, serum SP-D levels reflected the disease activity of pulmonary fibrosis [6].

Pulmonary surfactant protein D (SP-D) is considered as a candidate biomarker for the functional integrity of the lung and for disease progression, which can be detected in serum. The origin of SP-D in serum and how serum concentrations are related to pulmonary concentrations under inflammatory conditions is still unclear [7].

So this study aimed to determine the clinical significance of surfactant protein D (SP-D), as a marker for evaluating rheumatoid arthritis associated interstitial lung disease patients.

Patients and methods

This study was carried out at Chest and Internal Medicine Departments, Faculty of Medicine, Zagazig University in the period from March 2014 to February 2015.

Forty patients were recruited in this study. They were classified into 3 groups [Group 1: 18 patients (8 males and 10 females) had rheumatoid arthritis ILD; Group 2: 12 patients (4 males and 8 females) had rheumatoid arthritis without interstitial pulmonary disease; Group 3: 10 patients (7 males and 3 females) had interstitial pulmonary disease with no rheumatoid arthritis] in addition to 20 healthy control subjects (10 males and 10 females).

Exclusion criteria

1. Cases that were diagnosed with chronic obstructive pulmonary disease.
2. Cases that have had a respiratory tract infection during the last three months prior to enrollment.
3. Patients with hepatic, renal, cardiovascular diseases, diabetes mellitus, cancer and systemic inflammatory disorders other than rheumatoid disease.
4. Current smoker had been excluded from the study.
5. Any other rheumatologic disease had not been enrolled except for rheumatoid arthritis.

All patients of Group 3 were diagnosed with idiopathic pulmonary fibrosis (IPF), and had met American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines for the diagnosis and management of IPF [8].

Rheumatoid arthritis patients (Groups 1 and 2) were diagnosed according to the American College of Rheumatology/European League Against Rheumatism Classification Criteria for RA as the following: [9]

Score of ⩾6 points out of 10:

1. Joint involvement
   a. One large joint (0 points).
   b. Two to 10 large joints (1 point).
   c. One to three small joints (2 points).
   d. Four to 10 small joints (3 points).
   e. More than 10 joints (at least one small) (5 points).

2. Serology
   a. Negative rheumatoid factor (RF) and negative anti-cyclic citrullinated peptide antibody (aCCP) (0 points).
   b. Low positive RF or aCCP (> 3 times the upper limit of normal (2 points).

c. High positive RF or aCCP (> 3 times the upper limit of normal (3 points).

3. Acute phase reactants
   a. Normal C-reactive protein and normal erythrocyte sedimentation rate (0 points).
   b. Abnormal C-reactive protein or abnormal erythrocyte sedimentation rate (1 point).

4. Duration of symptoms
   a. < 6 weeks (0 points).
   b. $\geqslant 6$ weeks (1 point).

Group 1 patients had met the criteria of the American College of Rheumatology/European League Against Rheumatism Classification Criteria for RA [9] plus radiological evidence of interstitial lung disease by high resolution computed tomography (HRCT).

Written informed consents were obtained from all subjects.

All patients underwent a detailed symptom enquiry, physical examination and investigations including age, disease duration, CRP levels, rheumatoid factor, arterial blood gases, serum cyclic citrullinated peptide antibody, a chest radiograph (postero-anterior view), high resolution CT scan (HRCT) and pulmonary function tests as forced vital capacity (FVC).

The serum surfactant protein D (SP-D) levels were measured in the peripheral venous blood samples in all cases.

The serum samples, which were collected from the patients or healthy volunteers at their initial visits, were stored at -80 C until use and subsequently analyzed in a blinded fashion with regard to the patient's clinical status. Each serum sample was analyzed for the following:

1. Measurement of serum surfactant protein D (SP-D) was done by an enzyme-linked immunosorbent assay (ELISA). SP-D was measured by a commercially available ELISA kit (Endogen, MA).
2. Measurement of serum highly sensitive-CRP, rheumatoid factor (RF) and aCCP was done using an available commercial kit (ROCHE Diagnostics', COBAS 6000 auto analyzer, Germany). The measurement method used is based on a particle enhanced immunonephelometry and chemiluminescence assay and quality control is done before test measurement.

Spirometric values were measured using a spirometer. The best value of three maneuvers was expressed as a percentage of the predicted value and as absolute value. We concentrate on FVC values in this study.

Arterial blood gases analysis was done on APL autoanalyzer. Quality control was done before test measurement.

High resolution CT scan (HRCT) was done in all patients to reach to the diagnosis of ILD.

Statistical analysis

Differences in ages or the levels of the various serum markers between subject groups were analyzed using ANOVA one way test, Student's *t* test or Wilcoxon's rank sum test, if applicable. The levels of these serum markers were further analyzed using receiver operating characteristic (ROC) curves to determine the cut-off levels that resulted in the optimal diagnostic accuracy for each marker. Positive quantitative differences between groups were tested by the Chi-square test for goodness of fit or Fisher's exact probability test. The correlation coefficients for these markers were calculated using Spearman's correlation coefficient by rank. Significance was defined as $p < 0.05$.

Results

Table 1 shows descriptive study for age and sex in patients' groups and control group.

Table 2 shows that a highly statistical significant difference was found between patients' groups and control group regarding disease duration, serum aCCP, SP-D, serum CRP and RF being higher in patients' groups ($p < 0.01$). However FVC were significantly lower in patients' groups compared to the control group ($p < 0.05$).

Table 3 shows that a highly statistical significant difference was found between Group 1 vs Group 2 and Group 3 as regards SP-D ($p < 0.01$). However there was a significant difference between Group 2 vs Group 1 and Group 3 as regards FVC ($p < 0.05$). There was a very high statistical significant difference between Group 1 and Group 2 vs Group 3 as regards CRP, RF and aCCP.

Table 4 clarified that the comparison between control group vs all patient, control group vs Group 1, control group vs Group 3, Group 1 vs Group 2 and Group 2 vs Group 3 was highly significant ($p < 0.01$), while the comparison between control group vs Group 2 and Group 1 vs Group 3 was non significant.

Table 5 shows that there was a highly negative correlation between SP-D and FVC in Group 1 ($p < 0.01$) and a positive correlation between SP-D and CRP, RF, aCCP in Group 1 ($p < 0.05$), while there was a positive correlation between SP-D and RF in Group 2 ($p < 0.05$) and a highly negative correlation between SP-D and FVC in Group 3 ($p < 0.01$).

Fig. 1 shows receiver-operating characteristic (ROC) curve that was used for the assessment of the diagnostic performance of SP-D assay which revealed that the best cutoff value for discriminating rheumatoid patients with interstitial lung disease from those without interstitial lung disease was 219 ng/mL. At this value, SP-D had a diagnostic sensitivity of 94.2%, specificity 90%, negative predictive value 90%, positive predictive value 94.2% and efficiency 95%.

Discussion

ILD is one of the most severe complications in rheumatoid arthritis, and often carries a poor prognosis in spite of various therapies. The therapy for rheumatoid disease differs widely depending on the existence or activity of ILD. Therefore, it is important to evaluate the existence and severity of ILD when treating patients with rheumatoid arthritis. In general, chest radiography, lung function testing and blood gas analysis are usually used in the management of ILD in patients with rheumatoid arthritis [10]

In the present study, the serum SP-D level of rheumatoid arthritis patients with ILD (Group 1) was significantly elevated compared with those without ILD (Group 2). The incidence of

Table 1 Descriptive study for age and sex.

		Control group	Group 1	Group 2	Group 3	ANOVA	p
Age (mean ± SD)		42 ± 14	49 ± 11	51 ± 6	47 ± 12	1.89	0.14
						χ^2	p
Sex	Male n (%)	10 (50%)	8 (44.4%)	4 (33.3%)	7 (70%)	3.092	0.37
	Female n (%)	10 (50%)	10 (55.6%)	8 (66.7%)	3 (30%)		

χ^2: Chi-square test.

Table 2 Descriptive and comparative statistics of investigations in patients' group vs control group using student's t test for parametric data and Wilcoxon's rank sum test for non parametric data.

Parameter	Control group ($n = 20$) $\bar{X} \pm$ SD/ median (IQR)	All patients ($n = 40$) $\bar{X} \pm$ SD/median (IQR)	p
Disease duration months	0	39 (7–52)	$p < 0.01$
CRP (mg/L)	3.1 ± 1.5	32.4 ± 16.3	$p < 0.01$
RF (mg/L)	3.2 ± 6.2	46.4 ± 82.2	$p < 0.01$
FVC (% of expected)	95 (91–107)	64 (52–85)	$p < 0.05$
aCCP (ng/ml)	4.4 ± 2.1	23.2 ± 15.6	$p < 0.01$
SP-D (ng/ml)	31.0 ± 12.4	61.7 ± 122.6	$p < 0.01$

IQR (interquartile range).

Table 3 Descriptive and comparative statistics of investigations in patients' groups using ANOVA test for parametric data and Wilcoxon's rank sum test for non parametric data.

Parameter	Group 1 X ± SD/median (IQR)	Group 2 X ± SD/median (IQR)	Group 3 X ± SD/median (IQR)	p
Disease duration months	45 (21–52)	34 (22–48)	28 (12–36)	$p < 0.05$
CRP (mg/L)	42.2 ± 14.5	36.4 ± 12.8	21.2 ± 6.6	$p = 0.001$
RF (mg/L)	58 ± 24.2	49.4 ± 26.4	6.2 ± 4.1	$p < 0.001$
FVC (% of expected)	57 (52–72)	89 (78–99)	61 (48–88)	$p < 0.05$
aCCP (ng/ml)	26.2 ± 16.4	25.4 ± 18.2	7.2 ± 4.1	$p = 0.006$
SP-D (ng/ml)	204.2 ± 65	58.6 ± 14.6	91 ± 42.1	$p < 0.01$

decreased % FVC was significantly greater in patients with an elevated SP-D level than in those with a normal level. Furthermore, the serum SP-D level was inversely correlated with % FVC which is in agreement with the findings of Tracy et al. [11].

These results suggest that the serum SP-D level is a serum marker for ILD and reflects the severity of ILD in patients with rheumatoid arthritis. In this study, the diagnosis of ILD was made on the basis of the findings of chest radiography, computed tomography of the chest and lung function tests. However, there may be patients with minor respiratory crepitations, probably due to fibrosis, who do not have abnormal values in these examinations.

A highly significant difference was found between patients' group and control group regarding disease duration, serum aCCP, SP-D, serum CRP and RF being higher in patients' group ($p < 0.01$). However FVC was significantly lower in patients' group compared to the control group ($p < 0.05$). This reflects the sensitivity and specificity of this marker and the good choice of patient intensifying the exclusion criteria for our research, these results are in agreement with those of Sato et al. [12].

Table 4 Statistical comparison of serum SP-D between different studied groups using Wilcoxon's rank sum test.

Groups	z	p
Control vs all patients	−4.86	< 0.01
Control vs Group 1	−3.31	< 0.01
Control vs Group 2	−0.87	> 0.05
Control vs Group 3	−2.95	< 0.01
Group 1 vs Group 2	−2.42	< 0.01
Group 1 vs Group 3	−0.23	> 0.05
Group 2 vs Group 3	−2.56	< 0.01

The comparison between control vs all patient in SP-D levels was highly significant ($p < 0.01$) that indicates a high sensitivity and selectivity of the test. The comparison between control group vs rheumatoid arthritis with interstitial pulmonary disease SP-D levels was highly significant ($p < 0.01$), that totally agreed with most of other researches about SP-D.

The comparison between Rheumatoid arthritis with interstitial pulmonary disease group vs group of rheumatoid arthritis with no interstitial pulmonary disease in SP-D levels

Table 5 Correlation analysis between SP-D and different studied parameters in different studied groups using Spearman's rank correlation test.

Parameter	Group							
	Control		Group 1		Group 2		Group 3	
	r_s	p	r_s	p	r_s	p	r_s	p
Disease duration months	...	...	0.19	>0.05	0.26	>0.05	0.36	>0.05
CRP (mg/L)	0.25	>0.05	0.56	<0.05	0.49	>0.05	0.34	>0.05
RF (mg/L)	0.07	>0.05	0.57	<0.01	0.59	<0.05	0.13	>0.05
FVC (% of expected)	-0.18	>0.05	-0.65	<0.01	-0.12	>0.05	-0.72	<0.01
aCCP (ng/ml)	0.13	>0.05	0.59	<0.05	0.50	>0.05	0.17	>0.05
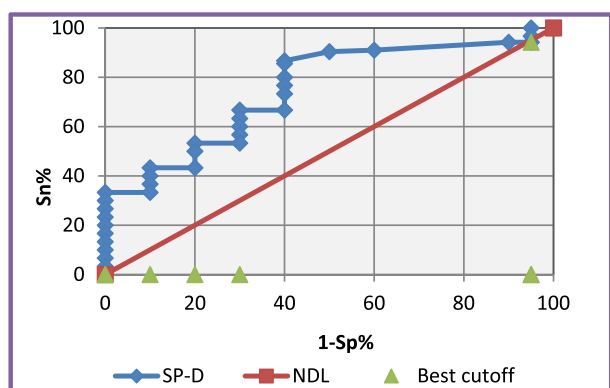
Figure 1 ROC curve analysis showing the diagnostic performance of SP-D for discriminating rheumatoid patients with interstitial lung disease from those without interstitial lung disease. *AUC:* area under cruve. *NDL:* non diagnostic line.

was highly significant $p < 0.01$. Similarly the comparison between the group with rheumatoid arthritis with no interstitial pulmonary disease vs the group with interstitial pulmonary disease with no rheumatoid arthritis was highly significant $p < 0.01$. This allows us to make a cut off in the ROC curve that was SP-D levels 219 ng/mL between rheumatoid arthritis with interstitial pulmonary disease group vs the group with rheumatoid arthritis with no interstitial pulmonary disease. This cut off is so essential for confirming interstitial pulmonary disease in rheumatoid patients making it a unique marker.

While the comparison between control vs the group with rheumatoid arthritis with no interstitial pulmonary disease and the group with Rheumatoid arthritis with interstitial pulmonary vs the group with interstitial pulmonary disease with no rheumatoid arthritis in SP-D levels was non significant ($p > 0.05$). This result was surprising as this omit the effect of rheumatoid activity in increasing the marker making the interstitial disease to take the upper hand in this marker this with disagree of Kristiansen et al. [10] that stated that rheumatoid arthritis increase this marker and illustrate that marker increase in disease activity that not confirmed in our study.

Assessment of the diagnostic performance of SP-D assay revealed that the best cutoff for discriminating rheumatoid patients with interstitial lung disease from those without interstitial lung disease was 219 ng/mL. At this value, SP-D had a

diagnostic sensitivity of 94.2%, specificity 90%, negative predictive value 90%, positive predictive value 94.2% and efficiency 95%, which we aimed in the study.

In conclusion, we have demonstrated the elevation of serum SP-D level in rheumatoid patients with interstitial lung disease. SP-D may be a useful marker for evaluating earlier ILD in patients with rheumatoid arthritis. However, this study was undertaken with a small patient population. Further studies are needed to clarify the usefulness of SP-D in rheumatoid arthritis patients.

References

[1] M. Fathi, J. Vikgren, M. Boijsen, et al, Interstitial lung disease in polymyositis and dermatomyositis: longitudinal evaluation by pulmonary function and radiology, Arthritis Rheum. 59 (2008) 677–685.

[2] B.W. Kinder, H.R. Collard, L. Koth, et al, Idiopathic nonspecific interstitial pneumonia: lung manifestation of undifferentiated connective tissue disease?, Am J. Respir. Crit. Care Med. 176 (2007) 691–697.

[3] T. Bongartz, C. Nannini, Y.F. Medina-Velasquez, et al, Incidence and mortality of interstitial lung disease in rheumatoid arthritis: a population-based study, Arthritis Rheum. 62 (6) (2010) 1583–1591.

[4] F. van Iwaarden, B. Welmers, J. Verhoef, H.P. Haagsman, L.M. G. van Golde, Pulmonary surfactant protein A enhances the host-defence mechanism of rat alveolar macrophages, Am. J. Respir. Cell Mol. Biol. 2 (1990) 91–98.

[5] H. Nagae, H. Takahashi, Y. Kuroki, et al, Enzyme-linked immunosorbent assay using F(ab')2 fragment for the detection of pulmonary surfactant protein D in sera, Clin. Chim. Acta. 266 (1997) 157–171.

[6] Y. Kuroki, H. Takahashi, H. Chiba, T. Akino, Surfactant proteins A and D: disease markers, Biochim. Biophys. Acta 1408 (1998) 334–345.

[7] M.G. Cosio, M. Saetta, A. Agusti, Immunologic aspects of chronic obstructive pulmonary disease, N. Engl. J. Med. 360 (2009) 2445–2454.

[8] G. Raghu, H.R. Collard, J.J. Egan, et alATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis, An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management, Am. J. Respir. Crit. Care Med. 183 (2011) 788–824.

[9] D. Aletaha, T. Neogi, A.J. Silman, et al, Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative, Ann. Rheum. Dis. 69 (9) (2010) 1580–1588 (published correction appears in Ann. Rheum. Dis. 69 (10) (2010) 1892).

[10] M. Kristiansen, M. Frisch, H.O. Madsen, P. Garred, S. Jacobsen, Smoking and polymorphisms of genes encoding mannose-binding lectin and surfactant protein-D in patients with rheumatoid arthritis, Rheumatol. Int. 34 (3) (2014) 373–380, http://dx.doi.org/10.1007/s00296-013-2904-z, Epub 2013 Nov 22.

[11] Tracy J. Doyle, Avignat S. Patel, Hiroto Hatabu, Mizuki Nishino, Detection of rheumatoid arthritis-interstitial lung disease is enhanced by serum biomarkers, ATS J. 191 (12) (2015) (June 15, 2015).

[12] S. Sato, M. Hanibuchi, A. Fukuya, Y. Yabuki, H. Bando, T. Yoshijima, H. Goto, H. Ogawa, Y. Nishioka, Idiopathic pleuroparenchymal fibrosis is characterized by an elevated serum level of surfactant protein-D, but not Krebs von den Lungen-6, Lung 192 (5) (2014) 711–717, http://dx.doi.org/10.1007/s00408-014-9599-0, Epub 2014 Jun 1.