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Prognostic significance of surfactant protein A, surfactant protein D, Clara cell protein 16, S100 protein, trefoil factor 3, and prostatic secretory protein 94 in idiopathic pulmonary fibrosis, sarcoidosis, and chronic pulmonary obstructive disease

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Abstract. *Background*: Identification of serum and bronchoalveolar lavage fluid (BALF) biomarkers may facilitate diagnosis and prognostication in various lung disorders. *Objective*: Serum and BALF levels of surfactant protein A (SP-A), surfactant protein D (SP-D), Clara cell protein 16 (CC16), S100 protein, trefoil factor 3 (TFF3), and prostatic secretory protein 94 (PSP94) were evaluated in 94 consecutive patients (idiopathic pulmonary fibrosis (IPF; n=18), sarcoidosis (n=25), chronic obstructive pulmonary disease (COPD; n=51)), and in 155 healthy controls. *Methods*: Biomarkers were measured at diagnosis and compared with disease characteristics. Both uniparametric and multiparametric analyses were used. *Results*: Seven significant correlations were found: 1) BALF PSP94 level correlated with prognosis of sarcoidosis (P=0.035); 2) BALF SP-D level with pulmonary functions in IPF (P=0.032); 3) BALF SP-D and TFF3 with IPF mortality (P=0.049 and 0.017, respectively); 4) serum TFF3 level with COPD mortality (P=0.006,); 5) serum SP-A with pulmonary functions impairment in IPF (P=0.011); 6) serum SP-D level was associated with HRCT interstitial score in IPF (P=0.0346); and 7) serum SP-A was associated with staging of COPD according to spirometry (P<0.001). Moreover, our analysis showed that some biomarker levels differed significantly among the diseases: 1) BALF SP-D level differed between sarcoidosis and IPF; 2) serum SP-A level differed among IPF, sarcoidosis, COPD and was also different from healthy controls; 3) serum S100A6, S100A11 levels differed among IPF, sarcoidosis, COPD from healthy controls 4) serum SP-D, CC16, TFF-3 levels distinguished IPF patients from healthy controls; and 5) serum CC16, TFF3, PSP94 distinguished COPD patients from healthy controls. Our study shows that some of selected biomarkers should have prognostic value in the analysed lung disorders. On the other hand, these biomarkers do not appear to be unequivocally suitable for differential diagnosis of these disorders. *(Sarcoidosis Vasc Diffuse Lung Dis 2016; 33: 224-234)*

Key words: surfactant protein A, surfactant protein D, Clara cell protein 16, S100 protein, trefoil factor 3, prostatic secretory protein 94, idiopathic pulmonary fibrosis, sarcoidosis, chronic pulmonary obstructive disease

Introduction

Biomarkers are defined as characteristics that are objectively measured and evaluated as indicators of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (1). Various biomarkers have been tested in many pneumological disorders, including idiopathic

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pulmonary fibrosis (IPF) (2), sarcoidosis (3), and chronic obstructive pulmonary disease (COPD) (4). There are currently no biomarkers broadly accepted and established for routine clinical application in pneumology. Therefore, novel and possibly more specific biomarkers should be tested. Surfactant protein A (SP-A), surfactant protein D (SP-D), Clara cell protein 16 (CC16), S100 protein, trefoil factor 3 (TFF3), and beta microseminoprotein/prostatic secretory protein 94 (PSP94) belong to these potentially suitable biomarkers. SP-A and SP-D are mainly synthesized in type II pneumocytes (5,6), and CC16 in Clara cells 16 but also in other extrapulmonary epithelial cell. CC16 is a protein with molecular weight of approximately 16 kd (7). S100 proteins are calcium-binding proteins. This protein complex was termed "S100" because of its solubility in 100% ammonium sulfate solution (8,9). PSP94, an unglycosylated protein of 94 amino acids, is one of the predominating proteins in the secretion of the human prostate gland. PSP is a member of immunoglobulin binding factor family that binds human IgG (10, 11, 12). TTF3 is a member of the trefoil factor family, which includes three members: TFF1 also called pS2, TFF2 or spasmolytic peptide (SP) and TFF3 or intestinal trefoil factor (ITF) (13, 14, 15).

Serum and bronchoalveolar lavage fluid (BALF) levels of SP-A, SP-D, CC16, S100, TFF3, PSP94 could indicate worse prognosis of IPF, sarcoidosis and COPD. Moreover, PSP94 and TFF3 have never been evaluated in BALF in these disorders. Therefore, the aim of this study was to evaluate the diagnostic and prognostic value of SP-A, SP-D, CC16, S100A6, S100A11, PSP94, TFF3 levels (measured in both BALF and serum) in IPF, sarcoidosis and COPD.

Patients and methods

Study subjects

All consecutive patients with IPF (n=18), sarcoidosis (n=25) and COPD (n=51), who were diagnosed and followed at the Department of Pulmonary Medicine of the University Hospital Brno, Czech Republic, since 2010, were included in this study. The IPF diagnosis was based on current American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines (typical histological or radiologic pattern of interstitial pneumonia) (2). The diagnosis of sarcoidosis was based on clinical examination, laboratory tests, pulmonary function test, radiologic methods, bronchoscopy with bronchoalveolar lavage, and histological finding of granuloma according to the World Association for Sarcoidosis and other Granulomatous Disorders (WASOG) (3). The diagnosis of COPD was defined according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (4).

Healthy subjects (n=155) served as a control group in order to assess the discriminative value of the serum markers. "Health" was confirmed using a medical checklist, the healthy subjects were blood donors. Clinical and demographic characteristics of the patient cohort are summarised in Table 1.

The study was approved by the independent ethic committee and all subjects signed informed consent.

Pulmonary function tests

Pulmonary function tests were performed in accordance with ERS recommendations (16, 17, 18). Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and diffusing capacity for carbon monoxide (DL_{co}) were measured. All values were stated as percentage of predicted value.

Radiologic imaging methods

High-resolution computed tomography (HRCT) alveolar (0-5) and interstitial scores (0-5) were assessed in IPF patients according to the criteria published by Gay *et al.* (19). Chest X-ray was used to divide sarcoidosis into 5 classical stages (0 - normal X-ray; I - presence of bilateral hillar lymfadenopathy; II - bilateral hillar lymfadenopathy and parechymal infiltrates; III - parenchymal infiltrates; IV - pulmonary fibrosis (3)).

Biomarkers measurement

Biomarkers were measured at diagnosis and compared with disease characteristics. Concentrations of SP-A, SP-D, CC16, S100A6, S100A11, PSP94 and TFF3 in serum and BALF samples were assessed by commercially available colorimetric sandwich ELISA kits (Biovendor - Laboratorni

Table 1. Basic clinical and demographic characteristics of analysed cohorts

	IPF	Sarcoidosis	COPD	Healthy controls
Number	18 patients	25 patients	51 patients	155 patients
Age, yr	68.5	38	65	40
F/M (gender)	8/10 patients	20/5 patients	15/36 patients	66/89 patients
Smoker	1 patients	3 patients	17 patients	ND
Non smoker	11 patients	21 patients	2 patients	ND
Ex-smoker	6 patients	1 patient	32 patients	ND
FVC% predicted (median)	68	91	51	ND
FEV1% predicted (median)	85	92	37	ND
DLco% predicted (median)	52	80.5	ND	ND
HRCT alveolar score - mean HRCT interstitial score - mean	1.16 2.5	ND	ND	ND
Stage of sarcoidosis (according to X-ray) – number patients	ND	I: 12, II: 12 III: 1, IV: 0	ND	ND
Stage of COPD according to GOLD – number patients	ND	ND	I: 3, II: 7, III: 7, IV: 34	ND
Spontaneous resolution	0	15 patients	0	NA
Deaths	6 patients	0	25 patients	ND

COPD – chronic obstructive pulmonary disease, DLco – diffuse lung capacity, F – female, FEV1% - forced expiratory volume in 1 s, FVC – forced vital capacity, GOLD (*Global Initiative for Chronic Obstructive lung disease* stage): stage I (FEV1/FVC< 0.70, FEV1≥ 80% normal), stage II (FEV1/FVC<0.70, FEV_1 50-79% normal), stage III (FEV1/FVC<0.70, FEV_1 30-49% normal), stage IV FEV1/FVC<0.70, FEV_1 <30% normal), HRCT – high resolution computed tomography, IPF- idiopathic pulmonary fibrosis, NA – not applicable; ND – not done

medicina, Brno, Czech Republic) according to the manufacturer's protocol. Serial dilution linearity testing was performed to select the proper BALF sample dilution with assay buffer for determination of S100A6 (1:500), S100A11 (1:10), PSP94 (1:100) and TFF-3 (1:5).

Statistical analysis

The One-Way ANOVA and Mann-Whitney tests were used to compare results among the analysed groups of patients. The Spearman rank test was used to assess correlations of two variables. Uniparametric and multiparametric analyses were performed as well. Differences were considered significant when P-value was < 0.05. Laboratory data were expressed as mean or median +/-SD (standard deviation). All statistical analyses were performed using SigmaPlot software, version 11.2 (Systat Software, Inc., San Jose, CA, USA).

Results

Biomarkers were analysed in 18 IPF patients, 25 sarcoidosis patients, 51 COPD patients, and 155 healthy controls. Clinical characteristics of this cohort are summarised in Table 1. Median age was 68.5 (49-79) years in IPF, 38 (26-68) years in sarcoidosis, and 65 (49-92) years in COPD.

Regarding evaluated biomarkers, seven significant correlations were found: 1) BALF PSP94 level correlated with prognosis of sarcoidosis (spontaneous disease resolution) (P=0.035); 2) BALF SP-D level correlated with pulmonary functions in IPF (P=0.032); 3) BALF SP-D and TFF3 with IPF mortality (P=0.049 and 0.017, respectively); 4) serum TFF3 level with COPD mortality (P=0.006,); 5) serum SP-A correlated with pulmonary functions impairment in IPF (P=0.011); 6) serum SP-D level was associated with HRCT interstitial score in IPF (P=0.0346); and 7) serum SP-A was associated with stages of COPD according to spirometry (0.011). Moreover, our analysis has showed that some biomarker levels differed significantly among the diseases: 1) BALF SP-D level differed between sarcoidosis and IPF; 2) serum SP-A level differed among IPF, sarcoidosis, COPD and was also different from healthy controls; 3) serum S100A6, S100A11 levels differed between IPF, sarcoidosis, COPD and healthy controls; 4) serum SP-D, CC16, TFF-3 levels distinguished IPF patients from healthy controls;

and 5) serum CC16, TFF3, PSP94 distinguished COPD patients from healthy controls. There were no statistically significant differences between BALF biomarkers´ levels and the extent of diseases in sarciodosis and IPF (measured by chest X-ray in sarcoidosis, and by alveolar and interstitial HRCT score in IPF), and between serum biomarkers´ levels and disease stage in sarcoidosis. Moreover, we found no statistically significant differences in the serum and BALF biomarkers´ levels in treated and untreated patients with IPF and sarcoidosis. All these results are summarized in Tables 2-11 and Figures 1-14.

Table 2. Serum concentrations of analysed markers in idiopathic pulmonary fibrosis (IPF), sarcoidosis, chronic obstructive pulmonary disease (COPD) and healthy controls

	SP-A ng/ml (median)	SP-D ng/ml (median)	S100A6 ng/ml (median)	S100A11 ng/ml (median)	CC16 ng/ml (median)	TFF-3 ng/ml (median)	PSP94 ng/ml (median)
IPF	98.107	623.1	123.376	24.29	17.615	1.248	17.203
Sarcoidosis	40.611	148.155	118.367	14.546	6.043	0.796	11.111
COPD	65.023	155.175	75.136	10.38	3.584	1.416	19.016
Healthy controls	17.426	155.119	54.869	5.597	5.821	0.681	13.492

CC16 – clara cells 16, COPD – chronic obstructive pulmonary disease, S100 A6, A11 – S100 protein SP-A – surfactant protein A, SP-D – surfactant protein D, IPF – idiopathic pulmonary fibrosis, PSP 94 – prostatic secretory protein 94, TFF3 – trefoil factor 3

Table 3. Prognostic significance of analysed serum markers in sarcoidosis in relation to disease remission. P value ≤ 0.05 is considered to be significant

	SP-A ng/ml (median)	SP-D ng/ml (median)	S100A6 ng/ml (median)	S100A11 ng/ml (median)	CC16 ng/ml (median)	TFF-3 ng/ml (median)	PSP94 ng/ml (median)
Resolution	46.817	138.071	122.139	12.686	6.377	0.883	9.521
Without resolution	37.483	153.475	118.367	18.835	6.763	0.654	12.714
P-value	0.206 (NS)	0.396 (NS)	0.642 (NS)	0.311 (NS)	0.741 (NS)	0.396 (NS)	0.106 (NS)

CC16 – clara cells 16, S100 A6, A11 – S100 protein SP-A – surfactant protein A, SP-D – surfactant protein D, NS – non significant, PSP 94 – prostatic secretory protein 94, TFF3 – trefoil factor 3

Table 4. Prognostic significance of serum markers in chronic obstructive pulmonary disease (COPD) in relation to pulmonary function tests. P-value ≤ 0.05 is considered to be significant

	SP-A ng/ml	SP-D ng/ml	S100A6 ng/ml	S100A11 ng/ml	CC16 ng/ml	TFF-3 ng/ml	PSP94 ng/ml
Deterioration	51.773	155.618	71.1	10.748	3.746	1.472	19.007
Stabilization	50.302	153.458	84.448	7.352	3.141	1.022	19.25
P-value	0.397 (NS)	0.828 (NS)	0.499 (NS)	0.384 (NS)	0.552 (NS)	0.423 (NS)	0.97 (NS)

CC16 – clara cells 16, S100 A6, A11 – S100 protein SP-A – surfactant protein A, SP-D – surfactant protein D, NS – non significant, PSP 94 – prostatic secretory protein 94, TFF3 – trefoil factor 3

Table 5. Prognostic significance of serum markers in chronic obstructive pulmonary disease (COPD) according to survival. P-value ≤ 0.05 is considered to be significant

	SP-A ng/m (median)	SP-D ng/ml (median)	S100A6 ng/ml (median)	S100A11 ng/ml (median)	CC16 ng/ml (median)	TFF-3 ng/ml (median)	PSP94 ng/ml (median)
Survivors	61.252	166.178	72.343	9.49	3.17	1.015	19.007
Non-survivors	70.271	132.447	85.851	10.38	3.902	1.743	20.085
P-value	0.346 (NS)	0.190 (NS)	0.468 (NS)	0.391 (NS)	0.258 (NS)	**0.006**	0.631 (NS)

CC16 – clara cells 16, S100 A6, A11 – S100 protein SP-A – surfactant protein A, SP-D – surfactant protein D, NS – non significant, PSP 94 – prostatic secretory protein 94, TFF3 – trefoil factor 3

Table 6. Prognostic significance of serum markers in relation to pulmonary function tests in idiopathic pulmonary fibrosis (IPF). P value ≤ 0.05 is considered to be significant

Pulmonary fuction tests	SP-A ng/m (median)	SP-D ng/ml (median)	S100A6 ng/ml (median)	S100A11 ng/ml (median)	CC16 ng/ml (median)	TFF-3 ng/ml (median)	PSP94 ng/ml (median)
Improvement	155.883	861.413	111.411	32.835	17.288	1.138	10.028
Deterioration	87.148	669.664	119.489	17.457	18.098	1.433	18.775
Stabilization	113.927	802.832	173.635	27.553	20.395	1.248	21.392
P-value	**0.011**	0.757 (NS)	0.569 (NS)	0.975 (NS)	0.916 (NS)	0.790 (NS)	0.242 (NS)

CC16 – clara cells 16, S100 A6, A11 – S100 protein SP-A – surfactant protein A, SP-D – surfactant protein D, NS – non significant, PSP 94 – prostatic secretory protein 94, TFF3 – trefoil factor 3

Table 7. Prognostic significance of serum analysed markers in relation to survival in idiopathic pulmonary fibrosis (IPF). P value ≤ 0.05 is considered to be significant

	SP-A ng/m (median)	SP-D ng/ml (median)	S100A6 ng/ml (median)	S100A11 ng/ml (median)	CC16 ng/ml (median)	TFF-3 ng/ml (median)	PSP94 ng/ml (median)
Survivors	115.286	718.869	123.376	24.29	16.822	1.263	17.576
Non-survivors	92.018	800.297	144.609	32.31	17.615	1.86	19.415
P-value	0.229 (NS)	0.714 (NS)	0.963 (NS)	0.426 (NS)	0.888 (NS)	0.119 (NS)	0.711 (NS)

CC16 – clara cells 16, S100 A6, A11 – S100 protein SP-A – surfactant protein A, SP-D – surfactant protein D, NS – non significant, PSP 94 – prostatic secretory protein 94, TFF3 – trefoil factor 3

Table 8. Prognostic significance of bronchoalveolar fluid (BALF) concentration of analysed markers in idiopathic pulmonary fibrosis (IPF) and sarcoidosis. P value ≤ 0.05 is considered to be significant

	SP-A ng/m (median)	SP-D ng/ml (median)	S100A6 ng/ml (median)	S100A11 ng/ml (median)	CC16 ng/ml (median)	TFF-3 ng/ml (median)	PSP94 ng/ml (median)
IPF	2796.929	536.542	127.036	136.94	420.524	2.576	72.51
Sarcoidosis	3862.516	1586.062	122.424	110.469	394.274	1.616	82.202
P-value	0.079 (NS)	**0.003**	0.0854 (NS)	0.758 (NS)	0.680 (NS)	0.150 (NS)	1.000 (NS)

CC16 – clara cells 16, COPD – chronic obstructive pulmonary disease, S100 A6, A11 – S100 protein SP-A – surfactant protein A, SP-D – surfactant protein D, IPF – idiopathic pulmonary fibrosis, NS – non significant, PSP 94 – prostatic secretory protein 94, TFF3 – trefoil factor 3

Table 9. Prognostic significance of bronchoalveolar lavage fluid (BALF) concentration of analysed markers in relation to sarcoidosis spontaneous remission. The results were considered significant if P ≤ 0.05

	SP-A ng/ml (median)	SP-D ng/ml (median)	S100A6 ng/ml (median)	S100A11 ng/ml (median)	CC16 ng/ml (median)	TFF-3 ng/ml (median)	PSP94 ng/ml (median)
Resolution	4048.203	1689.349	134.601	135.585	413.238	1.944	122.19
Without resolution	3716.619	1688.57	115.555	104.98	370.17	1.321	48.102
P-value	0.674 (NS)	0.999 (NS)	0.529 (NS)	0.258 (NS)	0.562 (NS)	0.286 (NS)	**0.035**

CC16 – clara cells 16, S100 A6, A11 – S100 protein SP-A – surfactant protein A, SP-D – surfactant protein D, NS – non significant, PSP 94 – prostatic secretory protein 94, TFF3 – trefoil factor 3

Table 10. Prognostic significance of bronchoalveolar lavage fluid (BALF) analysed markers in relation to pulmonary function tests in idiopathic pulmonary fibrosis (IPF). The results were considered significant if P ≤ 0.05

	SP-A ng/m (mean)	SP-D ng/ml (mean)	S100A6 ng/ml (mean)	S100A11 ng/ml (mean)	CC16 ng/ml (mean)	TFF-3 ng/ml (mean)	PSP94 ng/ml (mean)
Improvement	1944.641	1665.012	318.442	190.009	652.887	2,862	74.431
Deterioration	2339.068	637.811	81.696	50.784	333.822	4.964	63.978
Stabilization	2940.809	447.444	117.963	109.433	434.396	2.611	75.399
p-value	0.983 (NS)	**0.032**	0.185 (NS)	0.451 (NS)	0.122 (NS)	0.259 (NS)	0.930 (NS)

CC16 – clara cells 16, S100 A6, A11 – S100 protein SP-A – surfactant protein A, SP-D – surfactant protein D, NS – non significant, PSP 94 – prostatic secretory protein 94, TFF3 – trefoil factor 3

Table 11. Prognostic significance of bronchoalveolar lavage fluid (BALF) concentration of analysed markers in relation to survival in idiopathic pulmonary fibrosis (IPF). P value ≤ 0.05 is considered to be significant

	SP-A ng/ml (mean)	SP-D ng/ml (mean)	S100A6 ng/ml (mean)	S100A11 ng/ml (mean)	CC16 ng/ml (mean)	TFF-3 ng/ml (mean)	PSP94 ng/ml (mean)
Survivors	2773.261	226.325	209.981	181.461	462.253	2.732	73.83
Non-survivors	2009.703	620.605	67.868	47.353	337.064	6.026	67.884
P-value	0.482 (NS)	**0.049**	0.242 (NS)	0.206 (NS)	0.302 (NS)	**0.017**	0.815 (NS)

CC16 – clara cells 16, S100 A6, A11 – S100 protein SP-A – surfactant protein A, SP-D – surfactant protein D, NS – non significant, PSP 94 – prostatic secretory protein 94, TFF3 – trefoil factor 3.

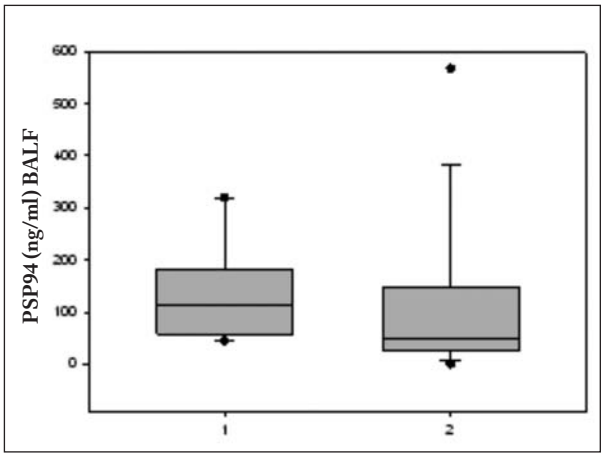

Fig. 1. Prostatic secretory protein (PSP94) in bronchoalveolar fluid (BALF) in sarcoidosis. Prognostics significance in relation to spontaneous resolution. 1 - no spontaneous resolution, 2 - spontaneous resolution

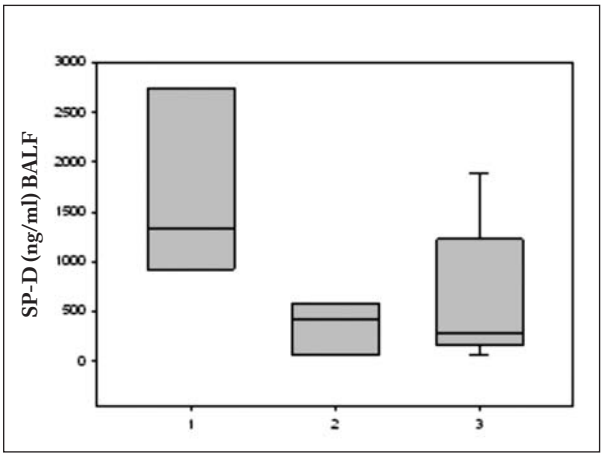

Fig. 2. Prognostic significance of surfactant protein D (SP-D) in bronchoalveolar fluid (BALF) in relation to pulmonary function tests in idiopathic pulmonary fibrosis (IPF). 1 - improvement, 2 - stable disease, 3 - deterioration

Discussion

Lung specific secretory proteins can be potential evaluative biomarkers that can help assess disease severity and progression, predict outcomes, and asses treatment effectiveness (20). SP-A and SP-D play an important role in pathogenesis of the some lung disorders (5). A complex of S100 proteins is involved in the regulation of many cellular processes such as cell division and differentiation (9). PSP94 is one of the predominating proteins in the human prostate gland secretion (10). However, the expression of PSP94 is not restricted to the prostate only. This protein has a widespread presence in the human body fluids. The highest concentrations of PSP94 were found in the respiratory tract secretion. Its concentrations in the

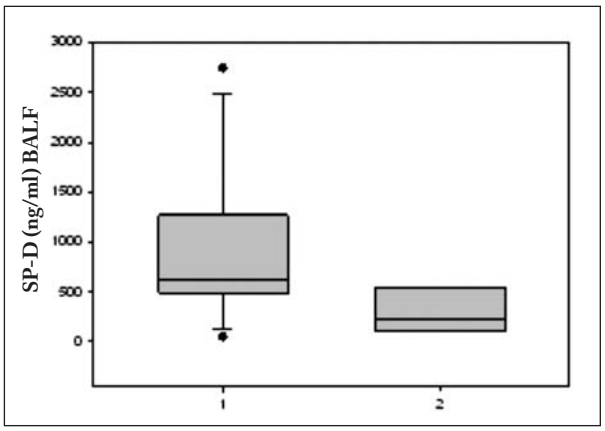

Fig. 3. Surfactant protein D (SP-D) in bronchoalveolar fluid (BALF) in idiopathic pulmonary fibrosis (IPF). Prognostics significance in relation to survival. 1 - alive, 2 - deceased

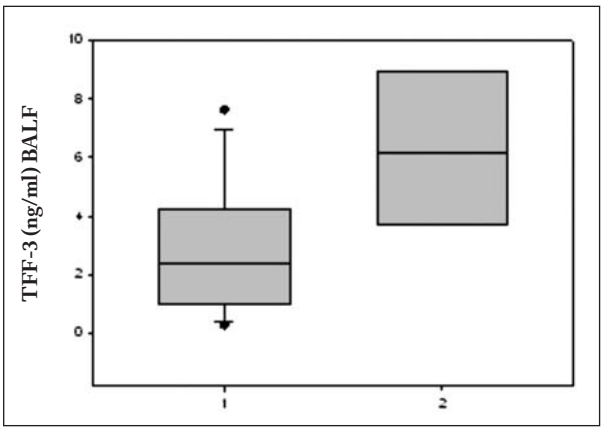

Fig. 4. Trefoil factor-3 (TFF-3) in bronchoalveolar fluid (BALF) in idiopathic pulmonary fibrosis (IPF). Prognostic significance in relation to survival. 1 - alive, 2 - deceased.

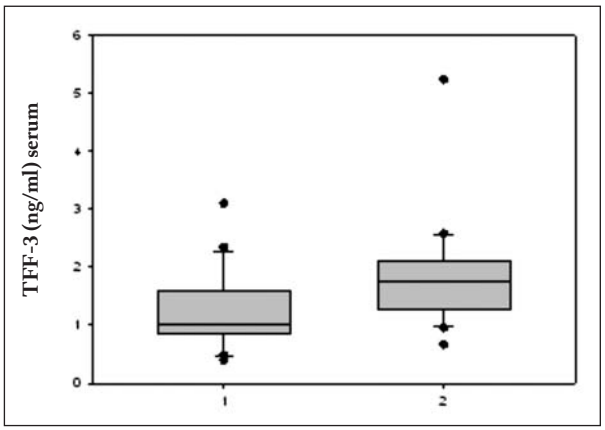

Fig. 5. Serum trefoil factor-3 (TFF-3) in chronic obstructive pulmonary disease (COPD). Prognostics significance in relation to survival. 1 - alive, 2 - deceased

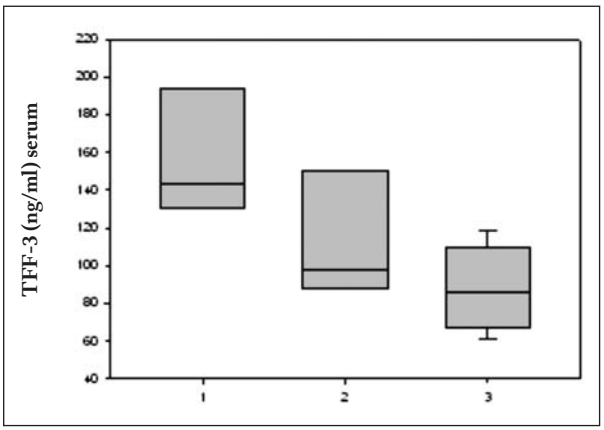

Fig. 6. Serum surfactant protein-A (SP-A) in idiopathic pulmonary fibrosis (IPF). Prognosis according to pulmonary function tests. 1 - improvement, 2 - stable disease, 3 - deterioration

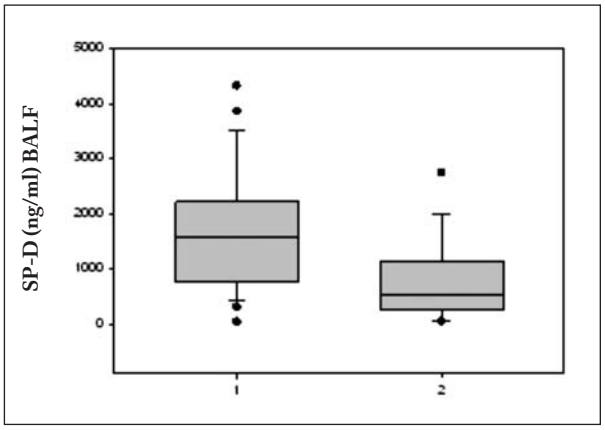

Fig. 7. Comparison of surfactant protein D (SP-D) levels in bronchoalveolar fluid (BALF) in sarcoidosis and idiopathic pulmonary fibrosis (IPF). 1 - sarcoidosis, 2 - IPF

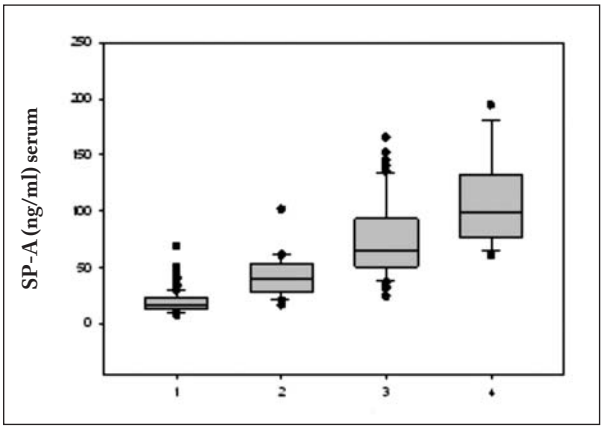

Fig. 8. Serum surfactant protein-A (SP-A) levels in analysed lung diseases and healthy controls. 1 - healthy controls, 2 - sarcoidosis, 3 - chronic obstructive pulmonary disease (COPD), 4 - idiopathic pulmonary fibrosis (IPF).

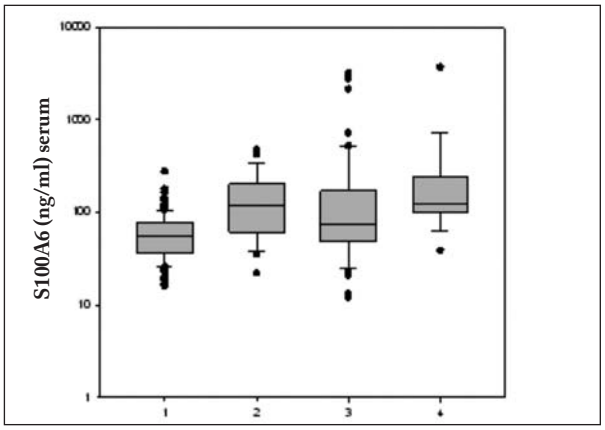

Fig. 9. Serum S100A6 levels in healthy controls (1), sarcoidosis (2), chronic obstructive pulmonary disease (COPD) (3), and idiopathic pulmonary fibrosis (IPF) (4)

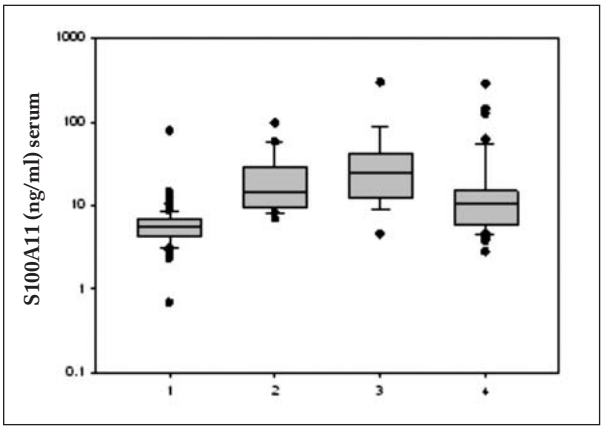

Fig. 10. Serum S100A11 levels in healthy controls (1), sarcoidosis (2), chronic obstructive pulmonary disease (COPD) (3), and idiopathic pulmonary fibrosis (IPF) (4)

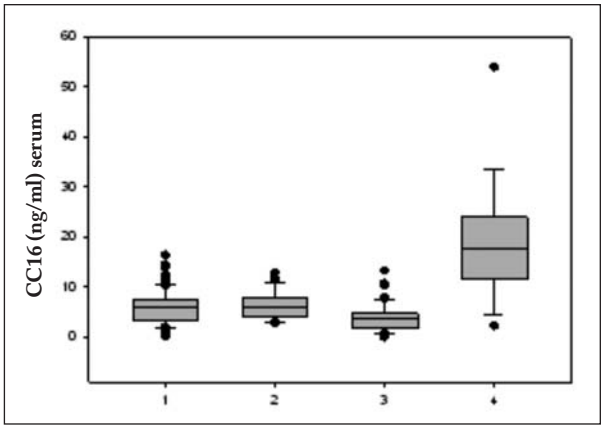

Fig. 11. Serum CC16 levels in healthy controls (1), sarcoidosis (2), chronic obstructive pulmonary disease (COPD) (3), and idiopathic pulmonary fibrosis (IPF) (4)

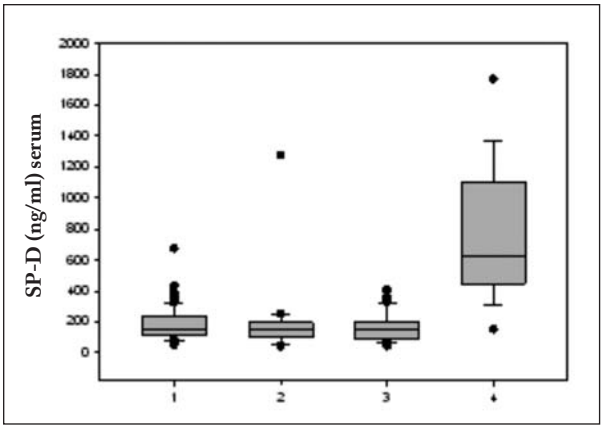

Fig. 12. Serum SP-D levels in healthy controls (1), sarcoidosis (2), chronic obstructive pulmonary disease (COPD) (3), and idiopathic pulmonary fibrosis (IPF) (4)

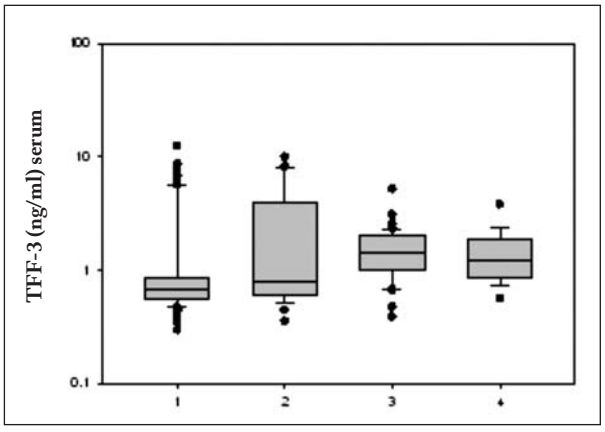

Fig. 13. Serum TFF-3 levels in healthy controls (1), sarcoidosis (2), chronic obstructive pulmonary disease (COPD) (3), and idiopathic pulmonary fibrosis (IPF) (4)

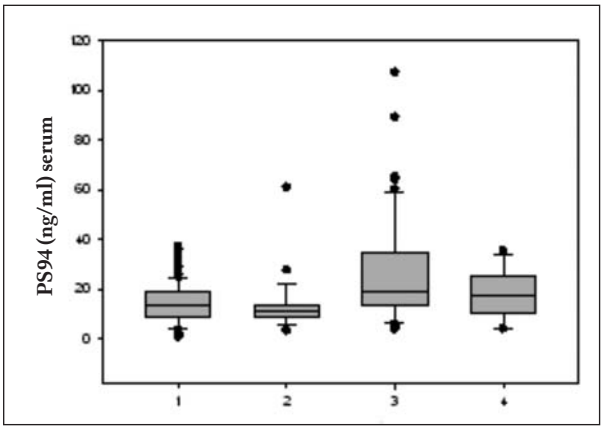

Fig. 14. Serum PSP94 levels in healthy controls (1), sarcoidosis (2), chronic obstructive pulmonary disease (COPD) (3), and idiopathic pulmonary fibrosis (IPF) (4)

tracheobronchial fluid are even comparable to that in the seminal fluid. PSP94 is also localized in the cilia of the ciliated epithelium of respiratory tract (11, 12). The TFF protein family is produced by mucin-secreting epithelial cells and play a crucial role in mucosal defense and healing. Although TFFs have been involved in the protection of the gastrointestinal tract against mucosal damage, their oncogenic potential has been extensively reported, including their role in cell proliferation, apoptosis, migration and invasion and angiogenesis (13, 14). TFF1 and TFF3 are expressed and secreted by normal and inflamed airways (15).

No specific prognostic markers for IPF, sarcoidosis and COPD have been identified to the date. There are currently no biomarkers broadly accepted and established for routine clinical application in pneumology, except for Japan, where Krebs von den Lungen-6 (KL-6), SP-A and SP-D are used in clinical practise as markers in diagnostics of interstitial lung diseases, although their lack of specifity for IPF has been acknowledged (20). As stated previously, identification of serum and BALF biomarkers may facilitate diagnostics and prognostication of various lung disorders, may distinguish between lung diseases and may help to indentify treatment responders.

Increased leakage of SP-A, SP-B and CC16 from the air spaces into the circulation occurs in many respiratory conditions. Although increased serum SP-A, SP-D, CC-16, S100 levels are most likely the result of increased secretion and/or leakage of these molecules across the alveolar-capillary membrane, it cannot be ruled out that there are other cells in the circulation that secrete some biomarkers (6, 21). Some clinical studies have suggested a predictive role of SP-A, SP-D and KL-6 for interstitial lung diseases (22). Serum SP-A was found at significantly higher levels in IPF than non-specific interstitial pneumonia (NSIP), sarcoidosis, and COPD (22). Significantly elevated serum SP-A and BALF SP-D have also been observed in our IPF patients compared to sarcoidosis.

Kunitake et al. found that SP-D, but not SP-A levels were significantly increased in pulmonary sarcoidosis compared with controls. SP-D levels probably reflect the damage to epithelial cells or release of these markers from these cells due to the inflammatory response (23). In our study, BALF SP-D differed between sarcoidosis and IPF but we did not confirm any relationship between SP-A or SP-D and staging of sarcoidosis.

Serum SP-A and SP-D levels appears to be highly predictive for survival of IPF patients (24, 25). A study Honda et al. showed that measurement of serum SP-D can provide an easily identifiable and useful clinical marker for the diagnosis of IPF and can predict the disease activity (25). Increased serum SP-A level was observed as a strong and independent predictor of early mortality among IPF patients (26). Song et al. compared plasma levels of matrix metalloproteinase-7 (MMP-7), KL-6 antigen, SP-A, and SP-D with a clinical course of 118 IPF patients. The combination of three markers (MMP-7, KL-6 and SP-A) predicted mortality of IPF compared with clinical parameters (27). In our study, BALF SP-D was associated with survival in IPF unlike BALF SP-A. Takahashi et al. demonstrated that SP-A and SP-D concentrations correlated significantly with the extent of alveolitis (a reversible change), whereas there was no correlation with fibrosis progression (an irreversible change). The SP-D concentration, unlike that of SP-A, was also related to the extent of parenchymal collapse and the rate of deterioration per year in pulmonary function (28). IPF patients with higher serum levels of SP-A and SP-D had a worse 3-year survival rate (29). In our cohort, a relationship between some markers and the extent of IPF was also observed. We detected significant correlation between serum SP-D and HRCT interstitial score (the extent of pulmonary fibrosis), and between BALF SP-D, serum SP-A and pulmonary function parameters in IPF patients.

A study of Hara et al. found that S100A9 levels were significantly higher in BALF in patients with IPF than in patients with other interstitial lung diseases and healthy volunteers (30). In our cohort, we found no prognostic significance of S100 (namely S100A6, A11) proteins in evaluated disorders. However S100 levels differed between these diseases and healthy controls.

Published data suggest that smokers, and especially current smokers, exhibit significantly reduced BALF SP-D and phospholipids compared to nonsmokers (31). BALF and serum SP-D levels were indicated as markers related to smoking, airway obstruction, and disease state (32). Changes in serum SP-D levels correlated well with changes in health status over a 3 month period in patients with severe

COPD (33, 34). Serum CC-16 levels were decreased in COPD and there was a positive correlation with disease severity (FEV1 decline over time) (35). In our study, we found only positive correlation of CC-16 levels with serum SP-A in COPD patients divided according to pulmonary function tests (GOLD I-IV stages).

PSP94 and TFF3 have not been evaluated yet in IPF, sarcoidosis and COPD. These markers play an important role mainly in oncology. The oncogenic potential of TFF3 has been reported. This protein is involved in cell proliferation, apoptosis, migration, invasion and angiogenesis (36). Fibrotic process that characterizes IPF is commonly considered to be a result of a recurrent injury to the alveolar epithelium followed by an uncontrolled proliferation of fibroblasts. Therefore, IPF might be considered a neoproliferative disorder of the lung, which exhibits several pathogenic features similar to cancer. Epigenetic and genetic abnormalities (altered cell-to-cell communications, uncontrolled proliferation, abnormal activation of specific signal transduction pathways) are biological hallmarks that characterize the pathogenesis of both IPF and cancer (37). Indeed, BALF TFF3 correlated with IPF mortality and serum TFF3 correlated with COPD mortality in our study. Moreover, BALF PSP94, as the only one of the analysed markers, correlated with prognosis of sarcoidosis according to spontaneous resolution.

We conclude that some of selected biomarkers should have a prognostic role in the analysed lung disorders. SP-A, SP-D, CC-16, S100 A6, A11, TFF3, PSP94 are markers that can be easily determined in serum and BALF. PSP94 seems to have a prognostic role in sarcoidosis, SP-D and TFF3 in IPF, and TFF3 in COPD patients. SP-A, SP-D, TFF3 reflect some aspects of pulmonary disease severity. On the other hand, these biomarkers do not appear to be unequivocally suitable for differential diagnosis of these disorders. Especially the very interesting results regarding to PSP94 and TFF-3 should be the aim of further studies.

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