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The role of biomarkers in the diagnosis and treatment follow-up of idiopathic pulmonary fibrosis

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Abstract. *Background and aim:* Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive lung disease of unknown cause with a poor prognosis. The aim of our study is to determine the role of Krebs von den Lungen-6 (KL-6), Matrix metalloproteinase (MMP)-7, Surfactant protein A (SP-A), Surfactant protein D (SP-D), vascular endothelial growth factor (VEGF) and periostin in the diagnosis of IPF and in the response monitoring of patients treated. *Method:* 47 IPF patients, 27 non-IPF interstitial lung disease (ILD) patients and 21 healthy individuals were included in the study. Demographic data, pulmonary function test- Diffusing capacity of the lung for carbon monoxide (PFT-DLCO) measurements, High-resolution computed tomography (HRCT) findings of the patients were recorded, and serum samples were taken. *Results:* While periostin and SP-A levels were not significantly different between IPF and non-IPF ILD, they were significantly higher in both IPF and non-IPF ILD compared to healthy control group (p=0.002, p=0.006 for periostin and p=0.002, p<0.001 for SP-A, respectively). By receiver operating characteristic (ROC) analysis, the cut-off point for periostin to distinguish IPF is >594.5 pg/ml (sensitivity 72%, specificity 76%), while the cut-off point for SP-A is found >6.62 ng/ml (sensitivity 87.2%, specificity 57.1%). In the combined ROC analysis based on SP-A=6.62 ng/ml and periostin >634.6 pg/ml values, sensitivity was found to be 85% and specificity was 57%.Considering the correlation of forced expiratory volume in the first second (FEV_1)(%), forced vital capacity (FVC)(%), restriction and diffusion severities with biomarker levels in the 6th month of IPF patients treated, a correlation was detected between MMP-7 levels and restriction severities (p=0.020), between KL-6 levels and restriction and diffusion severities (p=0.002), and between SP-A levels and FVC(%)(p=0.006). *Conclusion:* It is thought that biomarkers SP-A and periostin may contribute significantly to the diagnosis of patients with IPF, and SP-A, MMP-7 and KL-6 levels may contribute significantly to treatment follow-up.

Key words: idiopathic pulmonary fibrosis, biomarkers, diagnosis, follow-up

Introduction

Idiopathic pulmonary fibrosis (IPF); It is a chronic progressive fibrotic lung disease of unknown etiology, frequently occurring in older adults, and characterized histopathologically or radiologically by the usual interstitial pneumonia (UIP) pattern (1). It is the most common and most severe type of idiopathic interstitial pneumonia (IIP). The annual incidence of IPF is reported to be 2.8-9.3 per 100,000, and the mortality rate is reported to be 13.36 per 100,000 (2, 3). IPF is a disease with a poor prognosis and the average life expectancy is 2-5 years (1).

Biomarkers are being studied in the early diagnosis of IPF, determining the prognosis and monitoring the response to treatment. KL-6, MMP-7, SP-A, SP-D, VEGF and periostin are among these.

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KL-6 is secreted by bronchial epithelial cells and mostly by type 2 pneumocytes. Various studies have shown that KL-6 serum level is significantly high in the diagnosis of ILD compared to healthy control groups (4). Surfactant protein A (SP-A) and surfactant protein D (SP-D) are lung-specific proteins. High serum levels of SP-A in patients with IPF were first detected in 1993 (5). Thomeer et al. showed that serum SP-A levels are more significant in differentiating IPF from other ILDs. In the same study, serum SP-A levels in patients with usual interstitial pneumonia (UIP) were found to be significantly higher than in patients with non-specific interstitial pneumonia (NSIP) (6).

MMP-7 is secreted from alveolar macrophages and epithelial cells in IPF patients. Although it was found in the lung tissue of IPF patients, it could not be detected in the lung tissue of healthy people (7). Argyris et al. compared the MMP-7 levels of 97 IPF patients and 41 healthy control groups and found higher MMP-7 levels in the IPF group than the control group. The difference between the groups was found to be statistically significant ($p<0.001$). Vascular endothelial growth factor (VEGF) is a glycoprotein released from alveolar epithelial cells, induces vascular permeability, and is an important regulator of angiogenesis. Hubbard et al. showed in their studies that there is a relationship between VEGF and IPF (8). Periostin is an extracellular matrix protein that contributes to the development of fibrosis in the lungs, heart and bone marrow and is released from bronchial epithelial cells in response to interleukin-13 (IL-13) (9). It has been shown that there is a relationship between serum periostin level and the increase in radiological fibrotic area and prognosis.

The purpose of our study; To determine the role of KL-6, MMP-7, SP-A, SP-D, VEGF and periostin in the diagnosis and treatment follow-up of IPF.

Material and Method

Patients

74 patients over the age of 18 diagnosed with ILD at XXX Faculty of Medicine Chest Diseases Clinic and 21 healthy individuals were included in the study as a control group. None of the patients were in acute exacerbation in our study. The study was conducted by obtaining written consent from all patients and the individuals in the control group. Approval for the study was received from Eskişehir Osmangazi University Faculty of Medicine Ethics Committee (Decision No: 04.02.2020/15).

The inclusion criteria for the patient group were determined as follows;

- The patient has a newly diagnosed interstitial lung disease.
- Not receiving medical treatment for interstitial lung disease
- The patient does not have a malignancy

The inclusion criteria for the control group were determined as follows;

- Being over 50 years old
- Not having any known disease
- No history of asbestos exposure or smoking

Group with ILD; According to the ATS/ERS/JRS/ALAT 2108 IPF diagnostic guide (10), patients with IPF and non-IPF ILD were divided into two groups. Diagnoses were finalized at the council attended by chest-radiology specialists and, when necessary, pathology and rheumatology specialists. Demographic data, clinical and physical examination findings, and laboratory findings of the patients were recorded. HRCT was performed on the patients before diagnosis. PFT and DLCO measurements were made. All patients were evaluated with clinical and serologic tests for differential diagnosis and were evaluated by the rheumatology department for connective tissue diseases.

Antifibrotic treatment (pirfenidone or nintedanip) was started in patients diagnosed with IPF. Patients who started antifibrotic treatment were followed up. A control HRCT was performed at the 6th month of treatment. HRCT findings at the time of diagnosis and at the 6th month were evaluated and compared by the same radiologist. Sixth-month HRCT findings were recorded as stable or progressive compared to the findings at the time of diagnosis. In the 6th month of treatment, PFT and DLCO measurements were repeated and restriction and diffusion severities were evaluated.

We evaluated visual ILD progression, focusing on the CT findings as follows:

1. volume loss of the lungs;
2. increased extent of lesions (reticulation and honeycombing);

3. progression of traction bronchiectasis and architectural distortion;
4. appearance of new lesion(s) such as consolidation.

We determined the ILD progression when any 2 of the 4 findings were found on follow-up CT (11). Infection was excluded in the patients clinically and laboratory.

Biomarker assays

Serum was separated from blood samples taken from patients with IPF, non-IPF diseases (at the time of diagnosis) and individuals in the healthy control group. Blood samples were taken again from patients diagnosed with IPF at the 6th month of treatment and follow-up. Serum samples were stored at -40 °C until assayed. KL-6, MMP-7, SP-A, SP-D, VEGF and periostin levels were measured in the ESOGÜ Faculty of Medicine, Department of Medical Biochemistry Laboratory using ELISA kits.

Statistical analysis

Data analysis was performed with IBM SPSS 21 program (IBM SPSS statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) Descriptive statistics of quantitative) variables were shown as mean±standard error or median (Q1-Q3), while qualitative (categorical) variables were given as frequency and percentage. Normality of quantitative variables was evaluated with Shapiro Wilk test. Independent sample t test and Mann-Whitney U test were used for two independent group comparisons where the data was normally and non-normally distributed, respectively. Kruskal Wallis test was performed for three independent group comparisons. Pairwise comparisons of significant Kruskal Wallis results were evaluated with Dunn test. The relationship between qualitative variables was evaluated with chi-square analysis (Fisher exact). Repeated measurements of biomarkers and pulmonary function tests results were assessed with two-way mixed analysis of variance (ANOVA) using general linear models for repeated measures procedure. The model included group and time as main effects and a group * time interaction effect term. Post hoc testing was performed only for significant interactions and was carried out using a simple effect analysis with Bonferroni adjustment. Linear relationship between quantitative variables was evaluated with Spearman correlation analysis. Receiver Operating Characteristic (ROC) curve was used to assess the predictive performance of the biomarkers. P values less than 0.05 were considered significant.

Results

There were 47 patients with IPF in the study group, 27 patients with non-IPF ILD, and 21 healthy individuals in the control group. Non-IPF ILD group; It consisted of 21 patients with NSIP, 3 with chronic hypersensitivity pneumonia, and 3 with lung involvement due to collagen tissue diseases. The average age of the IPF group patients included in the study was 68±7 years, the non-IPF ILD group patients' average age was 65±8 years, and the average age of the control group consisting of healthy individuals was 66±6 years (p=0.157). 39 (83%) of the IPF patients, 14 (51.9%) of the non-IPF ILD patients, and 11 (52.4%) of the healthy control group were men. Patients in the IPF group had a smoking history of 29.45±26.89 pack/year, and patients in the non-IPF ILD group had a smoking history of 13.30±21.55 pack/year. Demographic characteristics of the participants by groups are listed in Table 1.

There was no statistically significant difference between FEV_1, FVC, TLC, DLCO at diagnosis between IPF and non-IPF ILD patients (Table 2).

While periostin levels did not differ significantly between IPF and non-IPF ILD, they were significantly higher in both IPF and non-IPF ILD compared to the healthy control group (p=0.002, p=0.006, respectively).

While SP-A levels did not differ significantly between IPF and non-IPF ILD, they were significantly higher in both IPF and non-IPF ILD compared to the healthy control group (p=0.002, p<0.001, respectively).

There was no statistically significant difference between groups for SP-D, KL-6, VEGF and MMP-7 (Table 3). The comparison of biomarkers between all groups is seen in Figure 1.

The area under the curve (AUC), cut-off points and their sensitivity and specificity values for serum periostin and SP-A concentrations as biomarkers for the diagnosis of IPF were obtained by ROC analysis. The cut-off point for periostin to distinguish IPF was found to be >594.5 pg/ml, and according to this

Table 1. Demographic characteristics of the study group of patients.

Demographic variables	IPF (n= 47)	Non-IPF ILD (n= 27)	Control Group (n= 21)	p
Age ($\bar{X}$ ± SD)	68±7	65±8	66±6	0.157
Gender n(%) Male Female	 39 (83)[b] 8 (17)[b]	 14 (51.9)[a] 13 (48.1)[a]	 11 (52.4)[a] 10 (47.6)[a]	 **0.006**
Cigarette smoking status n(%) Never smoker Current smoker Ex-smoker	 11(23.4)[a] 11 (23.4)[a] 25 (53.2)[a]	 16 (59.3)[b] 4 (14.8)[a] 7 (25.9)[b]	 21(100)	 **0.008**
Comorbidity n (%) No Yes	 13 (27.7) 34 (72.3)	 6 (22.2) 21 (77.8)	 21(100)	 **0.001**
Symptoms n(%) Cough Shortness of breath	 26 (55.3) 40 (85.1)	 13 (48.1) 21(77.8)	 	 **0.001** **0.001**

Abbreviations: IPF: Idiopathic pulmonary fibrosis; IPF: non-idiopathic pulmonary fibrosis; ILD: interstitial lung disease (Non-IPF ILD); $\bar{X}$: Mean; SD: Standard Deviation. [a,b] There is no significant difference between groups with the same letter.

Table 2. Comparison of PFT, DLCO in patients with IPF and non-IPF ILD.

Variable	IPF (n= 47)	non-IPF ILD (n= 27)	p
FEV_1 (%)	81.7±16.66	81.15±20.26	0.543
FVC (%)	76.51±15.78	74.41±21.35	0.630
TLC (%)	61.79±16.24	65.89±15.03	0.281
DLCO (%)	56.60±20.37	63.96±16.82	0.116

Abbreviations: FEV_1: Forced Expiratory Volume in the first second; FVC: Forced Vital Capacity; TLC: Total Lung Capacity; DLCO: Diffusing capacity of the lung for carbon monoxide; IPF: Idiopathic pulmonary fibrosis; IPF: non-idiopathic pulmonary fibrosis; ILD: interstitial lung disease (Non-IPF ILD).

value, the sensitivity was 72% and the specificity was 76%. The cut-off point of SP-A to distinguish IPF was found to be >6.62 ng/ml, and according to this value, the sensitivity was 87% and the specificity was 57%. The AUC value obtained with the combined model for periostin and SP-A was 0.742 and was considered significant (p<0.001). In the combined ROC analysis based on SP-A=6.62 ng/ml and periostin >634.6 pg/ml values, the sensitivity was found to be 85% and the specificity was 57% (Table 4). The ROC curve of SP-A and periostin was shown in Figure 2.

Table 3. Comparison of biomarkers across all groups.

Biomarkers	IPF (n= 47) $\bar{X}$ ± SD Median(Q1-Q3)	Non-IPF ILD (n=27) $\bar{X}$ ± SD Median(Q1-Q3)	Control Group (n=21) $\bar{X}$ ± SD Median(Q1-Q3)	p
Periostin (pg/ml)	698.9±312.0[a] 641.5(584.0-708.9)	857.5±685.6[a] 630.4(565.3-722.9)	571.3±112.9[b] 588.8(529.4-594.4)	**0.001**
SP-A (ng/ml)	8.23±3.76[a] 7.5(6.9-8.3)	10.59±8.47[a] 7.8(7.1-8.7)	6.43±1.25[b] 6.4(5.6-7.2)	**0.001**
SP-D (ng/ml)	143.2±73.0 124.6(107.5-148.5)	186.3±198.1 130.0(112.9-151.2)	144.9±28.4 156.9(126.4-162.4)	0.530
KL-6 (U/ml)	154.1±68.5 138.9(127.7-162.5)	200.6±170.9 154.9(138.5-167.3)	153.1±32.8 160.3(124.7-170.0)	0.511
VEGF (pg/ml)	118.0±50.5 107.2(96.1-122.7)	147.8±111.9 118.1(99.6-129.8)	118.3±25.3 115.4(101.3-135.1)	0.174
MMP-7 (ng/ml)	2.3±1.0 2.1(1.9-2.5)	2.41±1.4 2.1(1.9-2.4)	2.3±0.5 2.3(2.0-2.5)	0.298

Abbreviations: SP-A: Surfactant protein A; SP-D: Surfactant protein D; MMP-7: Matrix metalloproteinase 7; VEGF: Vascular endothelial growth factor; KL-6: Krebs von den lungen-6; $\bar{X}$: Mean; SD: Standard Deviation. IPF: Idiopathic pulmonary fibrosis; IPF: non-idiopathic pulmonary fibrosis; ILD: interstitial lung disease (Non-IPF ILD).

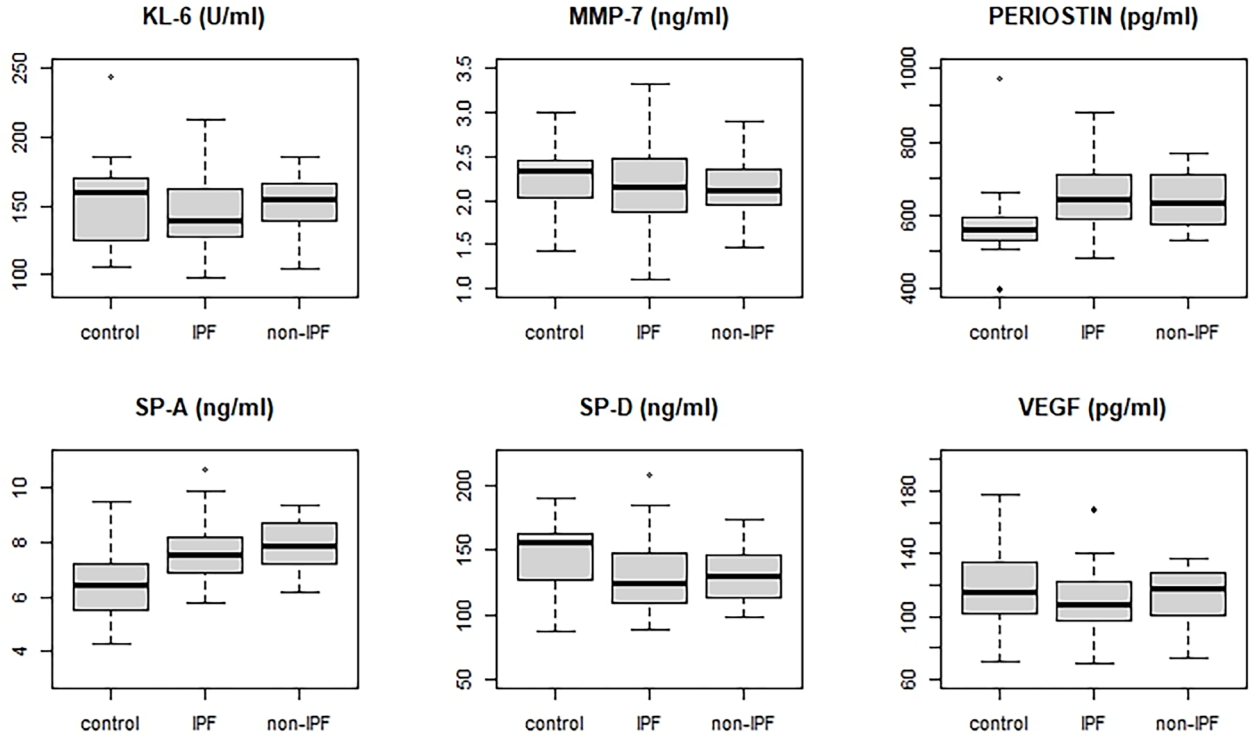

Figure 1. Comparison of biomarkers across all groups. Abbreviations: SP-A: Surfactant protein A; SP-D: Surfactant protein D; MMP-7: Matrix metalloproteinase 7; VEGF: Vascular endothelial growth factor; KL-6: Krebs von den lungen-6; IPF: Idiopathic pulmonary fibrosis; IPF: non-idiopathic pulmonary fibrosis; ILD: interstitial lung disease (Non-IPF ILD).

Table 4. ROC analysis of periostin and SP-A.

Biomarkers	AUC	p	CUT-OFF	SENSITIVITY (%)	SPECIFICITY (%)
Periostin(pg/ml)	0.753	p=0.001	594.5	72	76
SP-A(ng/ml)	0.763	p=0.001	6.62	87	57
Periostin(pg/ml) and SP-A(ng/ml)	0.742	p=0.002	634.6 and 6.62	85	57

Abbreviations: SP-A: Surfactant protein, AUC: Area Under Curve.

When the initial and 6th month biomarker levels of 32 patients diagnosed with IPF receiving antifibrotic treatment were compared, a statistically significant difference was observed between KL-6 levels (p=0.005). There was no statistically significant difference between SP-A, SP-D, periostin, MMP-7 and VEGF levels (Table 5). The patients' biomarker levels before treatment and 6th months of treatment are shown in Figure 3.

The restriction and diffusion severities of these 32 patients who received antifibrotic treatment after 6 months were compared with the HRCT subgroups. There was no significant difference between HRCT subgroups in terms of diffusion and restriction severity (p=0.644, p=0.295, respectively). When the relationship between the patients' PFT parameters and biomarker levels at the time of diagnosis and the 6th month of treatment is evaluated according to HRCT findings; The changes in SP-A levels, TLC (%) and DLCO (%) measurements from the beginning to the 6th month differ between the groups (p=0.034, p=0.020 and p=0.001, respectively). When the reason for this difference was investigated, a significant difference was observed between before and after measurements in the progression group (p=0.007, p=0.008 and p=0.016, respectively), but no significant change was observed in the stable group. When SP-D, periostin, KL-6, MMP-7, VEGF,

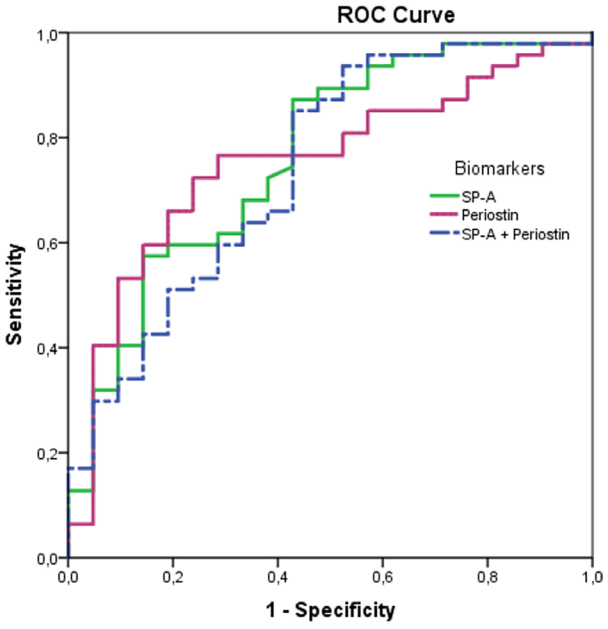

Figure 2. ROC curve of periostin and SP-A. Abbreviations: SP-A: Surfactant protein, receiver operating characteristic (ROC).

FEV_1 and FVC measurements were examined, the change from the beginning to the 6th month did not differ between the groups (Table 6).

The correlation between MMP-7 levels in patients receiving treatment and restriction severity after 6 months was statistically significant (p=0.020). These relationships were moderate and opposite. The correlation between KL-6 levels and restriction and diffusion severities measured after 6 months was statistically significant (p=0.002). These relationships were moderate and opposite. The correlation between SP-A levels and FVC (%) after 6 months was statistically significant (p=0.006). This relationship was also moderate and inverse. No statistically significant correlation was found between periostin, VEGF and SP-D levels and FEV1(%), FVC (%), restriction and diffusion severities measured after 6 months (p>0.05). The correlation of FEV1(%), FVC (%), restriction and diffusion severities with biomarker levels in patients with IPF after 6 months is shown in Table 7.

Table 5. Biomarker levels of IPF group patients before treatment and 6th month of treatment.

Biomarkers	Before treatment $\bar{X}$ ± SD Median(Q1-Q3)	6th month of treatment $\bar{X}$ ± SD Median(Q1-Q3)	p
SP-A(ng/ml)	8.50±5.42 7.46(6.68-8.16)	8.31±3.87 7.63(7.10-8.66)	0.174
SP-D(ng/ml)	155.8±118.4 130.0(112.9-158.7)	143.0±69.5 122.1(110.7-156.0)	0.079
Periostin(pg/ml)	715.8±437.0 626.2(555.3-691.8)	676.5±324.9 636.7(571.1-683.7)	0.970
KL-6(U/ml)	167.1±105.1 150.4(128.5-167.3)	171.2±80.8 157.2(145.2-173.9)	**0.005**
MMP-7(ng/ml)	2.31±1.02 2.14(1.86-2.53)	2.19±0.98 1.96(1.84-2.30)	0.852
VEGF(pg/ml)	126.5±70.9 113.5(98.8-127.9)	114.3±43.3 110.1(97.3120.4)	0.701

Abbreviations: SP-A: Surfactant protein A; SP-D: Surfactant protein D; MMP-7: Matrix metalloproteinase 7; VEGF: Vascular endothelial growth factor; KL-6: Krebs von den lungen-6; $\bar{X}$: Mea; SD: Standard Deviation.

Discussion

In this study, we compared the values of 6 serum biomarkers (KL-6, MMP-7, SP-A, SP-D, VEGF and periostin) at diagnosis and follow-up in the IPF, non-IPF ILD and control groups. In the analyses, we showed that serum SP-A and periostin can clearly distinguish IPF and non-IPF ILD patients from healthy controls and their diagnostic potential. In addition, we showed that SP-A, MMP-7 and KL-6 levels may have a significant contribution to treatment follow-up. In our study, SP-A levels did not differ significantly between patients with IPF and non-IPF ILD when the groups with ILD were compared, on the other hand, we found them to be high in both IPF and non-IPF ILD compared to the control group (p=0.002, p<0.001, respectively). Our results were consistent with those of Hamai et al. (12). When ROC curve analysis was used to evaluate the sensitivity and specificity of SP-A concentration for

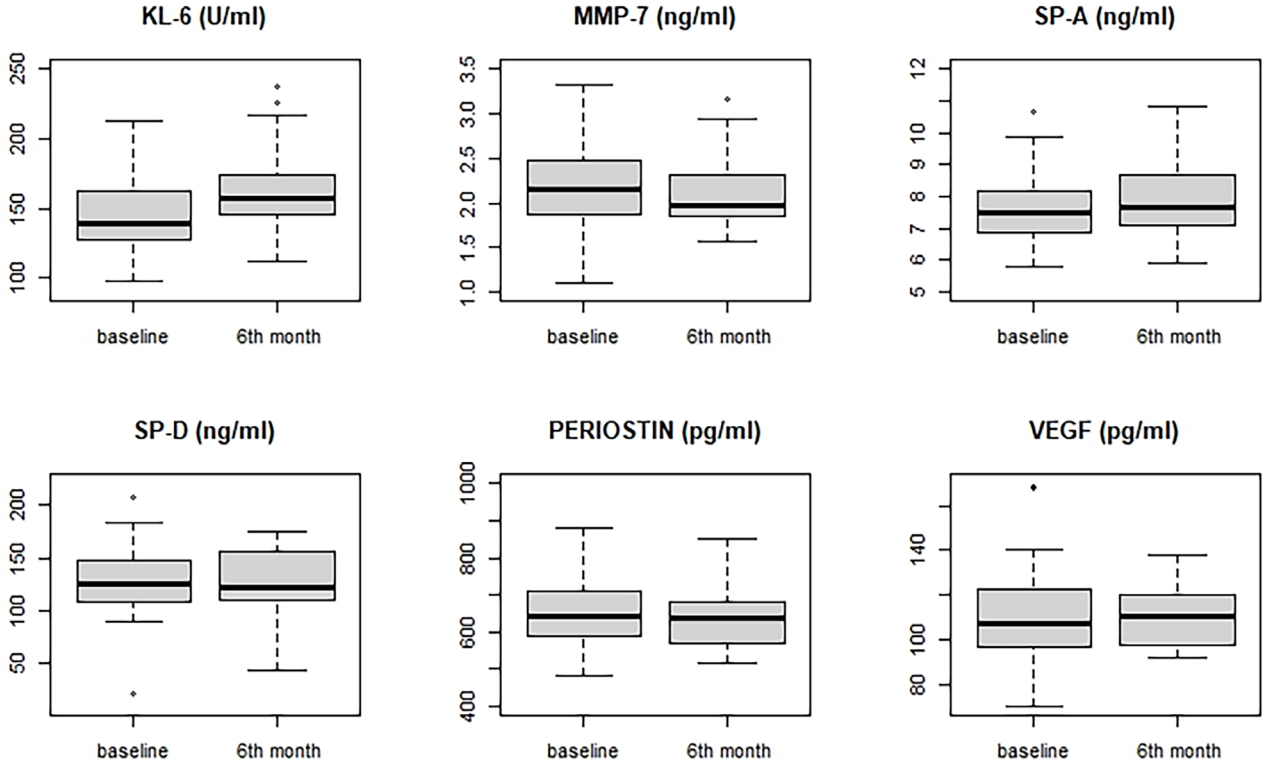

Figure 3. Biomarker levels of IPF group patients under treatment before treatment and 6th month of treatment. Abbreviations: SP-A: Surfactant protein A; SP-D: Surfactant protein D; MMP-7: Matrix metalloproteinase 7; VEGF: Vascular endothelial growth factor; KL-6: Krebs von den lungen-6.

the diagnosis of IPF, the cut-off point of SP-A to distinguish IPF was taken as >6.62 ng/ml, and according to this value, the sensitivity was 87% and the specificity was found as 57%. In our study, when periostin levels were compared between groups with IPF, no significant difference was found between IPF and non-IPF ILD, but they were found to be higher in both the IPF and non-IPF ILD groups compared to the control group (p=0.002, p=0.006, respectively). Our results were consistent with those of Okamoto et al. (13). When ROC curve analysis was used to evaluate the sensitivity and specificity of SP-A concentration for the diagnosis of IPF, the sensitivity was found to be 72% and the specificity was 76% when the predictive value of periostin to distinguish IPF was taken as >594.5 pg/ml.

In our study, no significant difference was detected in VEGF median values when these three groups were compared (p=0.174). In the study conducted by Masaru Ando et al., they compared the median values of serum VEGF in 41 IPF and 43 healthy volunteers, and no significant difference was found between the groups (14). In our study, no significant difference was found (p=0.530) when SP-D median values were compared in these three groups. However Barlo et al. compared the median SP-D values of 72 IPF and 305 healthy control groups and found that serum levels SP-D were significantly higher in patients than in controls (p<0.0001) (15). In our study, there was no significant difference in median KL-6 levels between these three groups (p=0.511). However, in study conducted by demirdöğen et al. serum KL-6 leves of serum 21 PFE and 26 IPF groups were compared, and serum KL-6 levels of the CPFE group were found to be significantly higher than the IPF group (p<0.001) (16). In our study, no significant difference was found in MMP-7 levels between these three groups (p=0.298). However, in the study conducted by Argyris et al. with a group of 97 IPF patients and 41 healthy control groups, MMP-7 levels were found to be higher in the IPF group compared to the control group (p <0.001) (17). In our study, patients with IPF were divided into two as progressive and stable according

Table 6. Examining the levels of biomarkers and respiratory function tests measured before treatment and 6th month of treatment in patients with IPF in HRCT subgroups.

Biomarkers	Stable (n=22)	Progression (n=10)	Time	Group	Group * Time
SP-A before treatment	8.65±0.996	6.33±0.587	**0.015**	0.234	**0.034**
SP-A 6th month of treatment	8.73±0.943	7.41±0.688			
SP-D before treatment	148.5±18.6	102.3±9.9	0.341	0.061	0.581
SP-D 6th month of treatment	160.0±16.2	105.4±8.7			
Periostin before treatment	731.2±80.3	556.1±52.5	0.485	0.070	0.260
Periostin 6th month of treatment	710.5±80.4	601.6±47.2			
KL-6 6th month of treatment	156.7±17.2	118.1±12.1	**0.001**	0.522	0.140
KL-6 6th month of treatment	185.7±19.5	139.3±11.6			
MMP-7 before treatment	2.34±0.24	1.83±0.18	0.984	0.153	0.634
MMP-7 6th month of treatment	2.37±0.23	1.80±0.15			
VEGF before treatment	123.1±12.7	92.9±8.2	0.542	0.233	0.165
VEGF 6th month of treatment	120.7±10.3	100.3±8.2			
FEV_1(%) before treatment	85.50±2.82	77.80±5.82	**0.010**	0.144	0.852
FEV_1(%) 6th month of treatment	80.00±2.00	72.00±7.00			
FVC (%) before treatment	80.64±3.00	75.00±5.10	0.001	0.214	0.519
FVC (%) 6th month of treatment	74.95±2.97	67.00±5.30			
TLC (%) before treatment	64.95±3.15	60.90±3.91	**0.036**	0.066	**0.020**
TLC (%) 6th month of treatment	65.50±3.06	51.40±2.88			
DLCO (%) before treatment	55.59±4.09	58.00±6.72	**0.001**	0.250	**0.001**
DLCO (%) 6th month of treatment	55.23±4.01	36.80±6.05			

Abbreviations: SP-A: Surfactant protein A; SP-D: Surfactant protein D; MMP-7: Matrix metalloproteinase 7; VEGF: Vascular endothelial growth factor; KL-6: Krebs von den lungen-6; FEV_1: Forced Expiratory Volume in the first second; FVC: Forced Vital Capacity; TLC: Total Lung Capacity; DLCO: Diffusing capacity of the lung for carbon monoxide.

Table 7. Correlation of FEV1, FVC, restriction and diffusion severities with biomarker levels of IPF group patients after 6 months.

	MMP-7	Periostin	SP-A	VEGF	KL-6	SP-D
FEV_1 (%)	r= 0.056 p= 0.760	r= -0.101 p= 0583	r= -0.274 p= 0.129	r= -0.081 p= 0.660	r= 0.183 p= 0.315	r= 0.064 p= 0.728
FVC (%)	r= 0.209 p= 0.252	r= -0.161 p= 0.380	**r= -0.473 p= 0.006**	r= -0.060 p= 0.746	r= 0.235 p= 0.196	r= 0.148 p= 0.419
Restriction Severity (TLC)	**r= -0.410 p= 0.020**	r= 0.307 p= 0.087	r= 0.340 p= 0.087	r= 0.007 p= 0.970	**r=-0.527 p= 0.002**	r=-0.202 p= 0.267
Diffusion Severity (DLCO)	r= -0.316 p= 0.078	r= 0.032 p= 0.864	r= 0.089 p= 0.627	r= -0.108 p= 0.556	**r=-0.522 p= 0.002**	r=-0.301 p= 0.094

Abbreviations: SP-A: Surfactant protein A; SP-D: Surfactant protein D; MMP-7: Matrix metalloproteinase 7; VEGF: Vascular endothelial growth factor; KL-6: Krebs von den lungen-6; FEV_1: Forced Expiratory Volume in the first second; FVC: Forced Vital Capacity; TLC: Total Lung Capacity; DLCO: Diffusing capacity of the lung for carbon monoxide.

to HRCT subgroups. When SP-A levels, TLC (%) and DLCO (%) values were examined, a difference was detected between the groups from the beginning to the 6th month (p=0.034, p=0.020, p=0.001, respectively). A significant difference was detected between before and after measurements of SP-A levels, TLC (%) and DLCO (%) in the progression group (p=0.007, p=0.008, p=0.016, respectively), but no significant change was observed in the stable group. There was no significant difference between the groups in KL-6, FEV_1 and FVC values. In the study conducted by Takumi et al. with patients with IPF who were treated and followed up, 49 patients included in the study were divided into "stable group (n=32)" (17 patients given pirfenidone; 15 patients given nintedanib) and "progressive group (n=17)." "(pirfenidone, 6; nintedanib, 11). The change in SP-A in the stable group was significantly lower than the change in the progression group (p<0.01) (18). In our study, when SP-D measurements were examined, no difference was found between the stable and progression groups from the beginning to the 6th month (p=0.581). In the study conducted by Ikeda et al., SP-D levels in IPF patients treated with pirfenidone predicted disease progression and prognosis (19).

In our study, a significant correlation was found between MMP-7 measured after 6 months and restriction severity (p=0.020). However, in the study conducted by Argyris et al. with 97 IPF and 41 healthy control groups, they found a negative correlation between plasma MMP-7 level and DLCO (r=-0.21, 95% CI: -0.42 to -0.04, p=0 .02) (17). In our study, the correlation between KL-6 levels measured 6 months later and DLCO was statistically significant (p=0.002). This relationship was moderate and inverse. However, no statistically significant correlation was found between SP-A and DLCO (p=0.627). In the study by Xue et al. in IIP, 19 of these patients had IPF, 23 had N-IPF and 27 had IPAF. In patients with IIP, serum levels of KL-6 and SP-A showed a significant negative correlation with DLCO (r= -0.36, -0.37, respectively; p < 0.05) (20). In our study, when VEGF measurements were examined, no difference was found between the stable and progression groups from the beginning to the 6th month (p=0.165). However, Simler et al previously reported that the change in VEGF over 6 months was significantly associated with the change in fibrosis score on HRCT (r=0.565, p=0.035) and was negatively associated with the change in FVC (r=-0.353, p=0.035) was detected. This suggests that IPF patients with progressive disease have higher basal VEGF concentrations than those who remain stable. In general, the baseline VEGF level may reflect the severity of IPF. It has been shown that serum VEGF levels of patients with IPF are correlated with HRCT interstitial score (21). In our study, when compared according to HRCT subgroups, the change in periostin values from the beginning to the 6th month did not differ. No correlation was found between periostin levels and FEV_1, FVC, restriction and diffusion intensities measured 6 months later in the IPF group patients. In the study conducted by Okamoto et al., the mean serum periostin levels were found to be 117.1±11.9 ng/ml in 37 IPF patients and 39.1±3 ng/ml in 66 healthy control groups, and were found to be associated with greater decreases in VC and DLCO (13). Another study showed that there is a relationship between serum periostin level and the increase in radiological fibrotic area and prognosis (22).

In conclusion, the findings we obtained in our study suggest that SP-A, periostin levels may have a significant contribution to the diagnosis of patients with IPF, and SP-A, MMP-7 and KL-6 levels may have a significant contribution to treatment follow-up. In our study, which coincided with the pandemic period, the limited number of patients and the fact that it was conducted in a single center were the limitations of our study. We think that the results of a multicenter study with larger patient series will shed light on the discussions on this subject.

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