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Comprehensive biomarker analysis of patients with idiopathic pulmonary fibrosis and interstitial lung disease with healthy individuals

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Abstract. – OBJECTIVE: Interstitial lung diseases (ILDs) are a group of diffuse parenchymal lung disorders that can be idiopathic [idiopathic pulmonary fibrosis (IPF)] or associated with other diseases and are characterized by varying degrees of inflammation and fibrosis with poor prognosis. Several indicators are essential in diagnosing these individuals and differentiating between IPF and ILD.

PATIENTS AND METHODS: The study involved 44 IPF patients, 22 ILD (non-IPF) patients, and 24 healthy people. We aimed to compare ILD (non-IPF) and IPF patient groups with each other and with healthy people in terms of interleukin (IL)-1, tumor necrosis factor-alpha (TNF-α), matrix metalloproteinase (MMP)-1, MMP-7, galectin (Gal)-3, IL-6, Krebs von den Lungen-6 (KL-6), total antioxidant status (TAS), total oxidant status (TOS), pyruvate kinase (PK), complete blood count (CBC), ferritin, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) features. Furthermore, it was intended to assess the patient groups in terms of visual semi-quantitative score (VSQS) (IPF alone), respiratory function tests (RFT), and 6-minute walk test (6MWT), also potential correlations between these tests and the previously indicated parameters.

RESULTS: MMP-1, MMP-7, Gal-3, IL-6, KL-6, forced vital capacity (FVC), % FVC, forced expiratory volume in 1 second (FEV1), % FEV1, TAS, TOS, and PK values significantly elevated in IPF and ILD. Weight, IL-1, MMP-1, MMP-7, Gal-3, IL-6, KL-6, % FVC, FEV1, % FEV1, eosinophil count, and % red blood cell distribution width (RDW) values differed between IPF and ILD. VSQS, 6MWT, and PK were substantially linked with MMP-1, MMP-7, Gal-3, IL-6, and KL-6 in IPF.

CONCLUSIONS: The factors investigated can be helpful in the diagnosis and distinction of IPF and ILD. In addition to focusing on the inflammatory environment in IPF and ILD patients, oxidant and antioxidant interactions must be studied.

Key Words:
Interstitial lung disease, Idiopathic pulmonary fibrosis, Biomarkers, Oxidative stress, Lung inflammation.

Introduction

General Information on the Subject

Interstitial lung diseases (ILDs) are a diverse collection of diffuse parenchymal lung disorders that can be idiopathic [idiopathic pulmonary fibrosis (IPF)] or related to other diseases, most notably connective tissue diseases (CTDs) (CTD-ILD) or sarcoidosis characterized by variable degrees of inflammation and fibrosis. If untreated, patients with IPF have a poor prognosis, with a median survival of 3-5 years[1]. Lung involvement is a frequent extra-articular consequence of CTDs such as systemic sclerosis (SSc), rheumatoid arthritis (RA), and dermatomyositis[2-4]. In addition, ILD is the leading cause of mortality in individuals with underlying RA and SSc and is a substantial contributor to morbidity[5,6]. However, managing ILDs is problematic because the individual prognosis is unpredictable. In addition, there is a wide range of disease histories ranging from stability or moderate progression over several years to fast deterioration, with severe exacerbations, which are significant causes of mortality, particularly in IPF[7]. Furthermore, IPF and CTD-ILD provide diagnostic problems, frequently resulting in delays that may increase morbidity and death. With the recent introduction of innovative and effective lung fibrosis therapies, it is crucial to identify patients with lung disease early and promptly identify those who will advance to severe lung disease[8-11].

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Importance of Biomarkers and Other Tests

A biomarker indicates normal biological processes, pathogenic processes, or reactions to an exposure or intervention, including therapeutic interventions[12]. The peripheral blood, airway, and lung parenchyma are sources of biomarkers that may help with diagnosis, outcomes, and therapy response in ILD. Peripheral blood is simple to acquire and requires little training beyond phlebotomy. Currently, several biomarkers are available in the literature for ILD and IPF diagnosis, therapy, and discrimination. Interleukin (IL)-1β plays an essential role in the pathogenesis of idiopathic pulmonary fibrosis. The production of IL-1β depends on caspase-1-containing multiprotein complexes called inflammasomes and the IL-1R1/myeloid differentiation primary response 88 (MyD88)/nuclear factor kappa B (NF-κB) pathway[13]. Researchers[14] discovered a strong connection between serum IL-6 levels and ILD progression/mortality in a large cohort of well-characterized patients with SSc-ILD with long-term functional follow-up after exploring a range of serum cytokines as potential biomarkers. Matrix metalloproteinase (MMP)-7 and MMP-1, according to the researchers[14], are overexpressed in the pulmonary microenvironment and distinguish IPF from other chronic lung illnesses. Increased MMP-7 concentrations may also suggest asymptomatic ILD and disease progression[15]. Tumor necrosis factor-alpha (TNF-alpha) and IL-6 production in rheumatoid arthritis and ILD patients evaluated in bronchoalveolar lavage specimens but not in blood indicates that alveolar macrophages are hyperreactive in these individuals, who may be sensitized as a result of the disease's inflammatory lung process[16]. Galectin (Gal)-3 is a profibrotic galactoside-binding lectin that plays a vital role in the pathophysiology of IPF and IPF exacerbations. It was demonstrated[17] to limit Gal-3 expression on bronchoalveolar lavage macrophages and, when combined, to reduce plasma indicators associated with IPF development. The Krebs von den Lungen-6 (KL-6) levels in ILD patients are unusually raised, although excessive KL-6 levels in healthy people or patients with other lung illnesses are uncommon. When the activity of ILD patients increases owing to an acute episode, the KL-6 level becomes even higher. As a result, KL-6 has a high value for ILD diagnosis and illness evaluation[18]. Literature studies aimed to evaluate the utility of neutrophil-to-lymphocyte ratio (NLR), the systemic immune-inflammation index (SII, neutrophil*platelet/lymphocyte), red blood cell distribution width (RDW), monocyte count, and other complete blood count (CBC) parameters as inflammation markers and prognostic factors in ILD and IPF[19-21]. Besides these valuable parameters, since IPF and ILD are inflammatory diseases, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and ferritin are also matter[22]. It is also essential to know about reactive oxygen species (ROS) produced from environmental exposures and inflammatory/interstitial cells mediating fibrosis. In addition to blood analyses, respiratory function tests (RFT) and the 6-minute walk test (6MWT) are also used to assess ILD and IPF complications[23]. Furthermore, the visual semi-quantitative score (VSQS) and several quantitative computed tomography (QCT) analyses have been carried out to determine IPF status[24].

Aim of the Study

This study aims to produce information about ILD and IPF that will contribute to the literature by using the parameters and tests we have mentioned. In addition, we aimed to identify patients in advance by controlling the inflammatory and oxidative environment that plays a role in the prognosis of ILD and IPF and to offer them a better quality of life by providing early treatment. Our study is the first research in the literature in which many parameters and tests related to the subject were investigated together.

Patients and Methods

Study Participants

This retrospective study was carried out between June 2019 and June 2022 in the Afyon Health Sciences University Faculty of Medicine Chest Diseases Clinic. A total of 90 people in three groups, including 44 IPF, 22 ILD (non-IPF) patients, and 24 healthy individuals, were included in the study. The hospital information system was used to acquire demographic information on the patients. Individuals with active infection, overlapping syndromes, or multiple autoimmune diseases were excluded when forming patient groups. Pregnants were omitted. The individuals selected for the groups were similar in gender. This study was performed in compliance with the Declaration of Helsinki and was approved by the Institutional Review Board of Afyon Health Sciences University Hospital.

Study Design

In terms of IL-1, TNF-α, MMP-1, MMP-7, Gal-3, IL-6, KL-6, total antioxidant status (TAS), total oxidant status (TOS), pyruvate kinase (PK), CBC, ferritin, ESR, and CRP characteristics, it was intended to compare ILD (non-IPF) and IPF patient groups with each other and with healthy persons. In addition, it was aimed to examine the patient groups in terms of VSQS (IPF only), RFT, and 6MWT and to investigate possible correlations of these tests with the previously mentioned parameters. Whole blood samples were drawn into tubes containing tripotassium (K_3) ethylene diamine tetraacetic acid (EDTA) for CBC and tubes containing 3.2% trisodium citrate solution (0.109 mol/L) for ESR. For the remaining biochemistry parameters, blood samples were taken into gel tubes without additives and then centrifuged at 1,500*g for 15 minutes to obtain serum and stored at -80°C until the study day. The working methodologies of the mentioned tests are given in Table I.

A multi-detector (160 Slice) computed tomography (CT) system was used to produce high-resolution computed tomography (HRCT) images (Aquilion Prime, Toshiba Medical Systems, Nasu, Japan). For the VSQS test of IPF patients, all HRCT pictures were taken with the lung window settings of -500 to -600 Hounsfield units (HU) and window width of 1,600 HU and were examined in consensus by two observers blinded to the clinical findings and RFT data. In addition, two radiologists (Observer 1 and Observer 2) performed the HRCT examination individually, using the semi-quantitative visual grading approach[25]. RFT and 6MWT[26] results were also recorded. In addition to 6MWT, first and last PO_2 saturation (Sat-First, Sat-End) and pulse (Pulse-First, Pulse-End) measurements were performed in patient groups. Besides, all participants' height and weight data were included in the study.

Statistical Analysis

Excel (Microsoft Inc, Redmont, WA, USA) was used to evaluate if the data were distributed normally. The differences in baseline statistics (mean, standard deviation, or frequency) of each variable between ILD, IPF, and healthy participants were calculated and compared. The Wilcoxon test was used to compare non-parametric group means, whereas the paired sample *t*-test was employed to compare parametric group means. The relationship between within-group parameters was analyzed by Pearson's and Spearman's correlation analyses. A p-value <0.05 were considered significant. Statistical analyses were assessed *via* SPSS 26 software (IBM Corp., Armonk, NY, USA).

Results

MMP-1, MMP-7, Gal-3, IL-6, KL-6, forced vital capacity (FVC), % FVC, forced expiratory volume in 1 second (FEV1), % FEV1, TAS, TOS, and PK values in IPF and ILD groups were substantially different from healthy individuals. Furthermore, compared to healthy people, the IPF group had a substantial difference in weight, IL-1, and TNF-α characteristics. There was a difference in weight, IL-1, MMP-1, MMP-7, Gal-3, IL-6, KL-6, % FVC, FEV1, % FEV1, eosinophil count, and % RDW values between the IPF and ILD groups. Moreover, VSQS, 6MWT, and PK were strongly associated with MMP-1, MMP-7, Gal-3, IL-6, and KL-6 in IPF patients. IPF patients were older than the other two groups. Other than the variables we provided, there was no difference between the groups regarding indicators. Table II shows descriptive data analysis. Table III contains group comparison statistics.

Discussion

Our extensive analysis is the first in the literature regarding the amount and diversity of biomarkers and indicators. First, in agreement with the literature, our study findings demonstrated higher MMP-1, MMP-7, Gal-3, IL-6, KL-6, IL-1, and TNF-α levels in IPF patients compared to healthy individuals[13-18]. Secondly, our study made a significant contribution to the literature by showing that TAS, TOS, and PK values, which are oxidative stress measures, were higher in IPF patients, which was consistent with our hypothesis. IPF may derive from the models and modes of action identified for induced pulmonary fibrosis. Environmental pollutants, mitochondrial/nicotinamide adenine dinucleotide phosphate (NADPH) oxidase depletion in inflammatory and lung target cells, and antioxidant defenses are thought to be the primary contributors to oxidative stress in pulmonary fibrosis[27]. These pathways' functions in the pathophysiology of IPF should be studied. Additionally, the correlations of VSQS and 6MWT measures with MMP-1, MMP-7, Gal-3, IL-6, and KL-6 in IPF patients demonstrate the significance of these parameters in the literature.

Table I. Working techniques for the stated parameters and employed devices.

Parameter	Sample	Methodology	Reactive	Device	Unit
Total Oxidant Status (TOS)	Serum	Colorimetric	TAS Reactive (Mega Tıp, Gaziantep, Turkey)	ChemWell (Awareness Technology Inc., Palm City, USA) Plate Reader	nmol H_2O_2 Equivalent/L
Total Antioxidant Status (TAS)	Serum	Colorimetric	TOS Reactive (Mega Tıp, Gaziantep, Turkey)	ChemWell (Awareness Technology Inc., Palm City, USA) Plate Reader	mmol Trolox Equivalent/L
Pyruvate Kinase (PK)	Serum	Colorimetric	Pyruvate Kynase Reactive (Mega Tıp, Gaziantep, Turkey)	ChemWell (Awareness Technology Inc., Palm City, USA) Plate Reader	Units/mg protein
IL-1β (Interleukin-1 Beta)	Serum	ELISA	eBioscience (San Diego, CA, USA)	ChemWell (Awareness Technology Inc., Palm City, USA) Plate Reader	pg/mL
TNF-α (Tumour Necrosis Factor-Alfa)	Serum	ELISA	eBioscience (San Diego, CA, USA)	ChemWell (Awareness Technology Inc., Palm City, USA) Plate Reader	pg/mL
MMP-1 (Matrix Metallo-proteinase-1)	Serum	ELISA	eBioscience (San Diego, CA, USA)	ChemWell (Awareness Technology Inc., Palm City, USA) Plate Reader	ng/mL
MMP7 (Matrix Metallo-proteinase-7)	Serum	ELISA	eBioscience (San Diego, CA, USA)	ChemWell (Awareness Technology Inc., Palm City, USA) Plate Reader	ng/mL
Gal-3 (Galectin-3)	Serum	ELISA	eBioscience (San Diego, CA, USA)	ChemWell (Awareness Technology Inc., Palm City, USA) Plate Reader	ng/mL
IL-6 (Interleukin-6)	Serum	ELISA	eBioscience (San Diego, CA, USA)	ChemWell (Awareness Technology Inc., Palm City, USA) Plate Reader	pg/mL
KL-6 (The Krebs von den Lungen-6)	Serum	ELISA	eBioscience (San Diego, CA, USA)	ChemWell (Awareness Technology Inc., Palm City, USA) Plate Reader	pg/mL
CBC (Complete Blood Count)	Whole Blood	Fluorescence Flow Cytometry	Sysmex (Sysmex Europe Company, Bornbarch, Germany)	XN-2000 (Sysmex Europe Company, Bornbarch, Germany)	Count/%
Ferritin	Serum	Immunoassay	Roche (Roche Diagnostics, Rotkreuz, Switzerland)	Roche Cobas 8000 (Roche Diagnostics, Rotkreuz, Switzerland)	µg/L
ESR (Erythrocyte Sedimentation Rate)	Whole Blood	Westergren Method	Sistat (Sistat Diagnostics, Çankaya, Turkey)	ESR-100 (Sistat Diagnostics, Çankaya, Turkey)	mm/hour
CRP (C Reactive Protein)	Serum	Spectro-photometric	Roche (Roche Diagnostics, Rotkreuz, Switzerland)	Roche Cobas 8000 (Roche Diagnostics, Rotkreuz, Switzerland)	mg/dL

Company for TAS, TOS, and PK reactive: Mega Tıp, Gaziantep, Turkey. Other device manufacturers are as follows: eBioscience (San Diego, CA, USA), Sysmex (Sysmex Europe Company, Bornbarch, Germany), Roche (Roche Diagnostics, Rotkreuz, Switzerland), Sistat (Sistat Diagnostics, Çankaya, Turkey), ChemWell (Awareness Technology Inc., Palm City, USA) Plate Reader.

Table II. Descriptive statistics of studied parameters.

Parameter	Group	Median	Mean	Std. Error of Mean	Std. Deviation	Minimum	Maximum
Age	IPF	66.000	66.432	1.287	8.538	49.000	85.000
Age	Healthy	56.500	57.792	1.334	6.534	45.000	73.000
Age	ILD	58.500	59.350	2.742	12.262	43.000	78.000
Height (cm)	IPF	165.000	165.045	1.296	8.594	149.000	180.000
Height (cm)	Healthy	164.500	165.083	1.478	7.241	154.000	175.000
Height (cm)	ILD	170.000	168.150	2.540	11.361	140.000	186.000
Weight (kg)	IPF	75.000	75.114	1.995	13.232	49.000	108.000
Weight (kg)	Healthy	85.500	82.833	2.900	14.209	52.000	116.000
Weight (kg)	ILD	92.500	95.750	8.200	36.670	55.000	205.000
VSQS	IPF	19.000	18.205	0.714	4.738	9.000	25.000
VSQS	Healthy	NaN	NaN	NaN	NaN	NaN	NaN
VSQS	ILD	NaN	NaN	NaN	NaN	NaN	NaN
IL-1β	IPF	41.950	44.993	4.759	31.569	5.100	135.000
IL-1β	Healthy	7.600	14.123	3.389	16.605	0.180	56.000
IL-1β	ILD	14.900	15.382	2.388	10.680	2.060	39.200
TNF-α	IPF	75.000	72.746	2.878	18.874	26.860	120.600
TNF-α	Healthy	18.150	19.854	1.031	5.052	12.550	28.800
TNF-α	ILD	29.400	48.796	19.460	87.028	10.000	413.600
MMP-1	IPF	16.500	14.523	0.849	5.630	4.000	21.000
MMP-1	Healthy	5.000	4.583	0.324	1.586	2.000	7.000
MMP-1	ILD	5.500	5.800	0.433	1.936	3.000	10.000
MMP-7	IPF	13.000	11.205	0.762	5.056	2.000	18.000
MMP-7	Healthy	2.000	2.417	0.208	1.018	1.000	4.000
MMP-7	ILD	5.000	4.900	0.397	1.774	2.000	8.000
Gal-3	IPF	15.250	15.034	0.512	3.399	10.200	20.600
Gal-3	Healthy	7.000	6.842	0.189	0.927	5.300	9.100
Gal-3	ILD	7.800	7.665	0.222	0.993	6.100	9.200
IL-6	IPF	2.500	2.515	227	1.504	100	5.500
IL-6	Healthy	75	108	19	95	8	350
IL-6	ILD	1.025	1.228	164	733	200	2.400
KL-6	IPF	2.975	2.872	231	1.530	450	5.750
KL-6	Healthy	290	256	39	192	10	510
KL-6	ILD	1.200	1.408	169	756	400	2.800
FVC	IPF	2.320	2.288	0.112	0.740	0.250	3.950
FVC	Healthy	2.955	2.786	0.153	0.750	1.200	4.450
FVC	ILD	2.375	2.019	0.216	0.966	0.280	3.550
%FVC	IPF	72.500	69.432	2.861	18.979	14.000	111.000
%FVC	Healthy	80.000	81.708	3.208	15.716	32.000	107.000
%FVC	ILD	57.000	55.500	5.259	23.520	10.000	92.000
FEV1	IPF	1.975	2.025	0.085	0.564	0.210	3.200
FEV1	Healthy	2.535	2.490	0.137	0.672	1.200	4.120
FEV1	ILD	1.575	1.653	0.187	0.834	0.280	3.070
%FEV1	IPF	79.500	76.545	2.858	18.956	16.000	110.000
%FEV1	Healthy	90.000	89.208	3.331	16.317	42.000	120.000
%FEV1	ILD	58.000	57.650	6.133	27.427	12.000	109.000
FEV1/FVC	IPF	90.000	88.409	1.505	9.980	61.000	109.000
FEV1/FVC	Healthy	91.000	89.708	1.356	6.643	77.000	100.000
FEV1/FVC	ILD	87.000	82.900	3.410	15.252	52.000	99.000
FEF25_75	IPF	2.475	7.939	3.669	24.339	0.630	121.000

Continued

Table II *(Continued)*. Descriptive statistics of studied parameters.

Parameter	Group	Median	Mean	Std. Error of Mean	Std. Deviation	Minimum	Maximum
FEF25_75	Healthy	2.980	3.135	0.196	0.959	1.430	5.640
FEF25_75	ILD	1.955	1.887	0.255	1.139	0.240	3.860
SII	IPF	643.450	894.652	114.991	762.765	199.300	4,468.300
SII	Healthy	609.200	803.263	115.110	563.922	310.500	2,580.700
SII	ILD	658.050	1,217.130	327.507	1,464.655	237.000	5,043.000
NLR	IPF	2.750	3.495	0.373	2.477	0.800	13.400
NLR	Healthy	2.400	2.779	0.310	1.520	1.100	7.000
NLR	ILD	2.250	6.825	2.644	11.826	1.200	41.000
Neu#	IPF	6,245	6,590	0,453	3,003	2,560	14,610
Neu#	Healthy	5,115	5,850	0,553	2,710	2,840	12,660
Neu#	ILD	4,920	5,901	0,864	3,862	3,120	20,930
Neu%	IPF	64.550	65.143	1.715	11.374	39.300	90.700
Neu%	Healthy	62.400	62.487	1.963	9.616	47.000	82.900
Neu%	ILD	61.750	62.615	3.889	17.392	6.500	93.900
Lym#	IPF	2,150	2,274	0,149	0,986	0,500	4,920
Lym#	Healthy	2,020	2,367	0,211	1,034	1,060	5,830
Lym#	ILD	2,040	1,893	0,186	0,831	0,140	2,990
Lym%	IPF	23.800	24.061	1.400	9.287	7.200	50.500
Lym%	Healthy	26.800	26.817	1.874	9.182	11.600	43.800
Lym%	ILD	27.000	23.146	2.369	10.594	2.130	39.300
Mono#	IPF	0,660	0,745	0,046	0,305	0,100	1,730
Mono#	Healthy	0,555	0,741	0,105	0,515	0,390	2,680
Mono#	ILD	0,645	0,703	0,092	0,410	0,220	2,140
Mono%	IPF	8.150	7.793	0.352	2.338	2.000	11.400
Mono%	Healthy	7.550	7.717	0.416	2.037	3.600	14.200
Mono%	ILD	8.000	7.975	0.479	2.142	3.600	11.200
Bas#	IPF	0,030	0,041	0,005	0,033	0,000	0,140
Bas#	Healthy	0,040	0,052	0,011	0,054	0,000	0,250
Bas#	ILD	0,035	0,048	0,010	0,043	0,000	0,180
Bas%	IPF	0.300	0.414	0.051	0.335	0.000	1.400
Bas%	Healthy	0.500	0.575	0.073	0.359	0.000	1.500
Bas%	ILD	0.500	0.525	0.075	0.337	0.000	1.200
Eos#	IPF	0,175	0,256	0,035	0,233	0,000	1,000
Eos#	Healthy	0,105	0,237	0,068	0,334	0,000	1,570
Eos#	ILD	0,100	0,126	0,023	0,103	0,000	0,360
Eos%	IPF	2.200	2.575	0.341	2.259	0.080	10.200
Eos%	Healthy	1.350	2.404	0.497	2.436	0.200	8.400
Eos%	ILD	1.300	1.730	0.364	1.629	0.000	5.400
Hb	IPF	14.500	14.123	0.268	1.778	9.700	17.600
Hb	Healthy	14.150	13.667	0.375	1.838	8.800	16.100
Hb	ILD	14.550	13.845	0.670	2.997	7.700	18.300
Hct	IPF	43.750	43.461	0.795	5.273	30.100	53.500
Hct	Healthy	43.400	42.304	0.973	4.767	31.300	48.500
Hct	ILD	44.650	43.065	1.706	7.630	27.400	55.500
PLT	IPF	256,000	251,545	10,982	72,847	88,000	505,000
PLT	Healthy	260,500	298,333	28,037	137,355	143,000	776,000
PLT	ILD	241,500	244,300	22,621	101,164	103,000	432,000
MPV	IPF	10.200	10.140	0.133	0.870	8.600	12.000
MPV	Healthy	10.050	10.129	0.232	1.135	7.500	12.300

Continued

Table II *(Continued)*. Descriptive statistics of studied parameters.

Parameter	Group	Median	Mean	Std. Error of Mean	Std. Deviation	Minimum	Maximum
MPV	ILD	10.000	9.988	0.179	0.716	8.900	11.600
%RDW	IPF	13.300	13.788	0.220	1.444	11.900	18.800
%RDW	Healthy	13.350	14.379	0.664	3.252	12.000	28.100
%RDW	ILD	14.350	15.615	0.700	3.133	12.700	22.300
RDW-SD	IPF	43.100	43.907	0.633	4.152	36.800	55.800
RDW-SD	Healthy	42.750	44.462	1.562	7.655	40.300	79.200
RDW-SD	ILD	45.450	46.555	1.627	7.275	35.000	63.600
Ferritin	IPF	83.710	174.560	39.363	208.288	14.470	735.200
Ferritin	Healthy	99.060	262.538	101.660	419.155	18.610	1.391.000
Ferritin	ILD	84.025	171.276	52.310	195.726	7.580	689.900
ESR	IPF	12.500	18.167	2.777	18.000	4.000	105.000
ESR	Healthy	8.000	14.773	3.034	14.229	4.000	62.000
ESR	ILD	20.500	26.833	5.585	23.695	2.000	79.000
MCV	IPF	89.550	88.395	0.996	6.610	67.800	107.600
MCV	Healthy	88.850	87.042	1.017	4.982	74.200	92.800
MCV	ILD	86.500	84.460	2.099	9.389	64.700	100.900
CRP	IPF	1.300	13.083	6.056	39.250	0.100	209.800
CRP	Healthy	1.900	4.727	1.290	6.049	0.000	18.800
CRP	ILD	0.950	4.001	1.524	6.464	0.100	24.800
TAS	IPF	14.860	16.855	0.820	5.437	11.620	33.090
TAS	Healthy	13.340	13.504	0.531	2.602	5.990	17.840
TAS	ILD	16.235	18.726	1.648	7.370	11.860	33.090
TOS	IPF	14.495	17.479	0.910	6.034	11.470	30.420
TOS	Healthy	13.360	13.188	0.657	3.219	5.670	19.160
TOS	ILD	14.385	18.298	1.629	7.287	9.790	30.420
PK	IPF	14.600	16.043	0.769	5.104	10.390	28.360
PK	Healthy	9.305	9.129	0.432	2.117	2.680	11.910
PK	ILD	14.190	15.892	1.388	6.209	7.960	28.360
Sat-First	IPF	94.000	93.159	0.519	3.444	84.000	100.000
Sat-First	Healthy	NaN	NaN	NaN	NaN	NaN	NaN
Sat-First	ILD	96.000	94.474	0.846	3.687	85.000	99.000
Sat-End	IPF	88.500	87.977	1.086	7.206	67.000	99.000
Sat-End	Healthy	NaN	NaN	NaN	NaN	NaN	NaN
Sat-End	ILD	93.000	90.316	1.393	6.074	76.000	99.000
Pulse-First	IPF	83.500	85.591	2.087	13.842	62.000	118.000
Pulse-First	Healthy	NaN	NaN	NaN	NaN	NaN	NaN
Pulse-First	ILD	82.000	82.158	2.485	10.833	60.000	99.000
Pulse-End	IPF	116.500	117.727	2.518	16.702	76.000	151.000
Pulse-End	Healthy	NaN	NaN	NaN	NaN	NaN	NaN
Pulse-End	ILD	115.000	116.211	3.625	15.803	84.000	149.000
6MWT	IPF	310.000	333.364	18.445	122.347	100.000	575.000
6MWT	Healthy	NaN	NaN	NaN	NaN	NaN	NaN
6MWT	ILD	430.000	409.263	31.750	138.394	150.000	556.000

NaN: Not applicable. VSQS; Visual semi-quantitative score, IL-1β; Interleukin-1 Beta, TNF-α; Tumour Necrosis Factor-Alfa, MMP-1; Matrix Metalloproteinase-1, MMP-7; Matrix Metalloproteinase-7, Gal-3; Galectin-3, IL-6; Interleukin-6, KL-6; Krebs von den Lungen-6, FVC; Forced Vital Capacity, %FVC; % Forced Vital Capacity, FEV1; Forced expiratory volume in 1 second, %FEV1; % Forced expiratory volume in 1 second, FEV1/FVC; FEV1/FVC ratio, FEF25_75; Forced expiratory flow, also known as mid-expiratory flow; at the rates at 25%, and 75% FVC are given, SII; Systemic immune-inflammation index, NLR; Neutrophile to lymphocyte ratio, Neu#; Neutrophile count, Neu%; Neutrophile percentage, Lym#; Lymphocyte count, Lym%; Lymphocyte percentage, Mono#; Monocyte count, Mono%; Monocyte percentage, Bas#; Basophile count, Bas%; Basophile percentage, Eos#; Eosinophile count, Eos%; Eosinophile percentage, Hb; Hemoglobine, Hct; Hematocrit, PLT; Platelets, MPV; Mean Platelet Volume, %RDW; % CV of Red Cell distribituion width, RDW-SD: SD of Red Cell distribituion width, ESR; Erytrocyte sedimentation rate, MCV: Mean corpuscular volume, CRP; C reactive protein, TAS; Total antioxidant status, TOS; Total oxidant status, PK; Pyruvate kinase, Sat-First; Initial oxygen saturation, Sat-End; End oxygen saturation, Pulse-First; Initial pulse, Pulse-End; End pulse, 6MWT; 6 minute walk test. IPF; Idiopathic pulmonary fibrosis, ILD; Interstitial Lung Disease.

Table III. Group comparison statistics.

Groups	IPF-Control		Control-ILD		IPF-ILD	
	t	*p*	t	*p*	t	*p*
Height (cm)	-0.018	0.985	-1.085	0.284	-1.208	0.232
Weight (kg)	-2.240	**0.028**	-1.591	0.119	-3.313	**0.002**
IL-1β	4.456	**< .001**	-0.292	0.772	4.075	**< .001**
TNF-α	13.422	**< .001**	-1.630	0.111	1.734	0.088
MMP-1	8.442	**< .001**	-2.293	**0.027**	6.725	**< .001**
MMP-7	8.395	**< .001**	-5.813	**< .001**	5.407	**< .001**
Gal-3	11.541	**< .001**	-2.840	**0.007**	9.477	**< .001**
IL-6	7.804	**< .001**	-7.422	**< .001**	3.626	**< .001**
KL-6	8.313	**< .001**	-7.206	**< .001**	4.048	**< .001**
FVC	-2.636	**0.010**	2.962	**0.005**	1.222	0.226
%FVC	-2.701	**0.009**	4.409	**< .001**	2.523	**0.014**
FEV1	-3.033	**0.003**	3.690	**< .001**	2.100	**0.040**
%FEV1	-2.760	**0.007**	4.728	**< .001**	3.199	**0.002**
FEV1/FVC	-0.571	0.570	1.977	0.055	1.724	0.090
FEF25_75	0.963	0.339	3.946	**< .001**	1.107	0.273
SII	0.515	0.609	-1.278	0.208	-1.161	0.250
NLR	1.288	0.202	-1.663	0.104	-1.799	0.077
Neu#	1.004	0.319	-0.051	0.960	0.777	0.440
Neu%	0.970	0.336	-0.031	0.976	0.694	0.490
Lym#	-0.363	0.718	1.653	0.106	1.504	0.138
Lym%	-1.174	0.245	1.231	0.225	0.349	0.728
Mono#	0.042	0.967	0.269	0.789	0.462	0.645
Mono%	0.135	0.893	-0.409	0.684	-0.296	0.768
Bas#	-1.015	0.314	0.274	0.786	-0.677	0.501
Bas%	-1.850	0.069	0.473	0.639	-1.230	0.223
Eos#	0.276	0.784	1.431	0.160	2.383	**0.020**
Eos%	0.289	0.773	1.055	0.297	1.501	0.138
Hb	0.999	0.321	-0.242	0.810	0.463	0.645
Hct	0.894	0.375	-0.404	0.689	0.241	0.810
PLT	-1.841	0.070	1.459	0.152	0.325	0.746
MPV	0.042	0.967	0.443	0.660	0.624	0.535
%RDW	-1.028	0.308	-1.276	0.209	-3.184	**0.002**
RDW-SD	-0.386	0.701	-0.923	0.361	-1.837	0.071
Ferritin	-0.940	0.352	0.749	0.460	0.049	0.961
ESR	0.767	0.446	-1.992	0.054	-1.551	0.126
MCV	0.876	0.384	1.166	0.250	1.928	0.058
CRP	0.989	0.327	0.366	0.716	0.972	0.335
TAS	2.840	**0.006**	-3.243	**0.002**	-1.138	0.260
TOS	3.235	**0.002**	-3.097	**0.003**	-0.471	0.639
PK	6.329	**< .001**	-5.008	**< .001**	0.102	0.919
Sat-First					-1.362	0.178
Sat-End					-1.236	0.221
Pulse-First					0.960	0.341
Pulse-End					0.336	0.738
6-Min-Test					-2.172	0.034

Group comparison statistics. Values with $p < 0.05$ are shown in bold. VSQS; Visual semi-quantitative score, IL-1β; Interleukin-1 Beta, TNF-α; Tumour Necrosis Factor-Alfa, MMP-1; Matrix Metalloproteinase-1, MMP-7; Matrix Metalloproteinase-7, Gal-3; Galectin-3, IL-6; Interleukin-6, KL-6; Krebs von den Lungen-6, FVC; Forced Vital Capacity, %FVC; % Forced Vital Capacity, FEV1; Forced expiratory volume in 1 second, %FEV1; % Forced expiratory volume in 1 second, FEV1/FVC; FEV1/FVC ratio, FEF25_75; Forced expiratory flow, also known as mid-expiratory flow; at the rates at 25%, and 75% FVC are given, SII; Systemic immune-inflammation index, NLR; Neutrophile to lymphocyte ratio, Neu#; Neutrophile count, Neu%; Neutrophile percentage, Lym#; Lymphocyte count, Lym%; Lymphocyte percentage, Mono#; Monocyte count, Mono%; Monocyte percentage, Bas#; Basophile count, Bas%; Basophile percentage, Eos#; Eosinophile count, Eos%; Eosinophile percentage, Hb; Hemoglobine, Hct; Hematocrit, PLT; Platelets, MPV; Mean Platelet Volume, %RDW; % CV of Red Cell distribituion width, RDW-SD: SD of Red Cell distribituion width, ESR; Erytrocyte sedimentation rate, MCV: Mean corpuscular volume, CRP; C reactive protein, TAS; Total antioxidant status, TOS; Total oxidant status, PK; Pyruvate kinase, Sat-First; Initial oxygen saturation, Sat-End; End oxygen saturation, Pulse-First; Initial pulse, Pulse-End; End pulse, 6MWT; 6 minute walk test. IPF; Idiopathic pulmonary fibrosis, ILD; Interstitial Lung Disease.

Even though these procedures are user-dependent[25,26], they aid in diagnosing IPF in a non-invasive and simple manner. Besides, in contrast to ILD patients and healthy people, IPF patients weighed less. Although the poor prognosis and considerable FVC drop in fast losing weight of IPF patients had been highlighted in the literature[28,29], this data was a discovery for which we could not find a scientific explanation.

Our research has added a new viewpoint to the literature regarding ILD patients. MMP-1, MMP-7, Gal-3, IL-6, KL-6, FVC, % FVC, FEV1, % FEV1, TAS, TOS, PK, and forced expiratory flow 25-75 (FEF25-75) indicators were considerably increased in ILD patients compared to healthy people in our comprehensive analysis. Moreover, in an unfathomable way, the ILD group was heavier than the healthy and IPF groups. Although we chose ILD patients as non-IPF, the majority of the indicators produced different outcomes in the ILD and IPF groups compared to healthy persons, which first perplexed us. We found significant changes in weight, IL-1, MMP-1, MMP-7, Gal-3, IL-6, KL-6, % FVC, FEV1, eosinophil count, and % RDW when we evaluated the two patient groups. Numerous possible IPF biomarkers have been identified to assess disease severity and prognosis, including KL-6, surfactant protein A/D (SP-A/D), matrix metalloproteinases, and osteopontin[30-32]. KL-6 is a common biomarker for the clinical therapy of ILDs in Japan. However, as various researchers[30,31] have pointed out, this molecule is more suited to assess disease behavior and prognosis than distinguishing ILDs. Researchers[33] also discovered that latent transforming growth factor (TGF)-binding protein-2 might influence the process of fibroblast-to-myofibroblast differentiation. The same working group contended that gremlin-1 was increased in fibrotic lungs, particularly in IPF, and that serum concentration measurements might help improve the diagnostic certainty of IPF *vs.* non-IPF ILDs[34]. Despite this knowledge, our study is notable for the amount and variety of characteristics that may be utilized to distinguish between IPF and ILD patients.

Although the specific mechanism is unknown, oxidative stress has been linked[35] to the etiology of pulmonary fibrosis. Advanced glycosylated end-products (AGE) are considered potential biomarkers. AGE is generated by combining glycation, oxidation, and/or carbonylation[36]. Advanced oxidation protein products (AOPP) are enhanced in several chronic inflammatory diseases with significant oxidative stress overload. High plasma levels of AOPP have been reported[37] in lung diseases. High TAS, TOS, and PK levels in IPF and ILD groups in our research findings confirmed earlier studies, allowing us to look at the issue in terms of different parameters.

We expected that the inflammatory environment caused by IPF and ILD would raise SII, NLR, CRP, ESR, ferritin, and some other CBC parameters. However, we found no difference in these metrics between patient groups and healthy participants. In addition, researchers discovered that SII and NLR measures did not significantly differ between IPF and ILD patients and healthy people in a study[38] with participants identical to the study group we developed. The findings of our investigation are compatible with the conclusions of this study. Therefore, we believe that the inflammatory environment in these disorders is inadequate or that our study parameters are lacking in this respect.

Bronchoalveolar lavage (BAL) eosinophilia may be a sign of progressive lung disease in individuals with IPF and pulmonary fibrosis linked with a collagen vascular abnormality (PF-CVD), according to the researchers[39]. We discovered that the eosinophil count in the CBC, rather than the BAL, may be utilized to distinguish between IPF and ILD. This might be because the processes that generate eosinophilia in BAL have a comparable impact on the blood. Furthermore, studies[40] indicate that the RDW is a commonly available laboratory test result that may give crucial, independent prognostic information at baseline and follow-up in IPF patients. Based on our findings, we also believe RDW can be utilized to distinguish between IPF and ILD.

Limitations

The study's one drawback is the small number of patients. Another limitation is that we did not deal with BAL samples. BAL samples can more accurately reflect lung tissue's inflammatory and oxidative status. One of the most significant limitations of the study is that we conducted the research on the subject samples all at once. Metrics that demonstrate the time-dependent adjustments that may be made to the parameters will be more helpful.

Conclusions

In summary, MMP-1, MMP-7, Gal-3, IL-6, KL-6, FVC, % FVC, FEV1, % FEV1, TAS, TOS, and PK values of IPF and ILD patients change significantly from healthy individuals. On the other hand, eosinophil count and % RDW can

be utilized to distinguish between IPF and ILD patients. The VSQS and 6MWT are non-invasive tests that aid in diagnosing IPF patients. The impact of NLR and SII on IPF and ILD is unknown. The ROS markers TAS, TOS, and PK, on the contrary side, may assist in the identification of both IPF and ILD patients. In addition to concentrating on the inflammatory environment in IPF and ILD patients, additional attention must be paid to the oxidant and antioxidant interactions. Studies with larger numbers of patients and control groups will be more enlightening.

Ethics Approval
This study was performed in compliance with the Declaration of Helsinki and was approved by the Institutional Review Board of Afyon Health Sciences University Hospital (2022/128).

Informed Consent
Informed consent was obtained from all individuals included in the study.

Conflict of Interest
All authors declare that there is no conflict of interest.

Data Availability
Data is available if requested.

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None.

Authors' Contributions
Aydın Balcı: conceptualization, methodology, formal analysis, ınvestigation, writing-original draft, writing-review & editing, visualization, project administrator; Muhammed Emin DÜZ: methodology, investigation, formal analysis, project administrator; Ayhan Vurmaz: conceptualization, methodology, formal analysis, investigation, writing-review & editing, visualization; Şule Çilekar: conceptualization, methodology, formal analysis, writing-review & editing, project administrator; Furkan Kaya: methodology, investigation, formal analysis, visualization.

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References

1) Elhai M, Avouac J, Allanore Y. Circulating lung biomarkers in idiopathic lung fibrosis and interstitial lung diseases associated with connective tissue diseases: Where do we stand? Semin Arthritis Rheum 2020; 50: 480-491.
2) Bongartz T, Nannini C, Medina-Velasquez YF, Achenbach SJ, Crowson CS, Ryu JH, Vassallo R, Gabriel SE, Matteson EL. Incidence and mortality of interstitial lung disease in rheumatoid arthritis: a population-based study. Arthritis Rheum 2010; 62: 1583-1591.
3) Schnabel A, Reuter M, Biederer J, Richter C, Gross WL. Interstitial lung disease in polymyositis 7 and dermatomyositis: clinical course and response to treatment. Semin Arthritis Rheum 2003; 32: 273-284.
4) Cutolo M, Kitas GD, van Riel PLCM. Burden of disease in treated rheumatoid arthritis patients: 10 going beyond the joint. Semin Arthritis Rheum 2014; 43: 479-488.
5) Singh N, Varghese J, England BR, Solomon JJ, Michaud K, Mikuls TR, Healy HS, Kimpston EM, Schweizer ML. Impact of the pattern of interstitial lung disease on mortality in rheumatoid arthritis: A systematic literature review and meta-analysis. Semin Arthritis Rheum 2019; 12: 358-365.
6) Hax V, Bredemeier M, Didonet Moro AL, Pavan TR, Vieira MV, Pitrez EH, da Silva Chakr RM, Xavier RM. Clinical algorithms for the diagnosis and prognosis of interstitial lung disease in systemic sclerosis. Semin Arthritis Rheum 2017; 47: 228-234.
7) Kennedy B, Branagan P, Moloney F, Haroon M, O'Connell OJ, O'Connor TM, O'Regan K, Harney S, Henry MT. Biomarkers to identify ILD and predict lung function decline in scleroderma lung disease or idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis 2015; 32: 228-236.
8) Silver KC, Silver RM. Management of Systemic-Sclerosis-Associated Interstitial Lung Disease. Rheum Dis Clin North Am 2015; 41: 439-457.
9) Raghu G, Rochwerg B, Zhang Y, Garcia CA, Azuma A, Behr J, Brozek JL, Collard HR, Cunningham W, Homma S, Johkoh T, Martinez FJ, Myers J, Protzko SL, Richeldi L, Rind D, Selman M, Theodore A, Wells AU, Hoogsteden H, Schünemann HJ; American Thoracic Society; European Respiratory society; Japanese Respiratory Society; Latin American Thoracic Association. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. Am J Respir Crit Care Med 2015; 192: 3-19.
10) Distler O, Highland KB, Gahlemann M, Azuma A, Fischer A, Mayes MD, Raghu G, Sauter W, Girard M, Alves M, Clerisme-Beaty E, Stowasser S, Tetzlaff K, Kuwana M, Maher TM; SENSCIS Trial Investigators. Nintedanib for Systemic Sclerosis-Associated Interstitial Lung Disease. N Engl J Med 2019; 380: 2518-2528.

11) Dempsey TM, Sangaralingham LR, Yao X, Sanghavi D, Shah ND, Limper AH. Clinical 35 Effectiveness of Antifibrotic Medications for Idiopathic Pulmonary Fibrosis. Am J Respir Crit 36 Care Med 2019; 200: 168-174.

12) Wu AC, Kiley JP, Noel PJ, Amur S, Burchard EG, Clancy JP, Galanter J, Inada M, Jones TK, Kropski JA, Loyd JE, Nogee LM, Raby BA, Rogers AJ, Schwartz DA, Sin DD, Spira A, Weiss ST, Young LR, Himes BE. Current Status and Future Opportunities in Lung Precision Medicine Research with a Focus on Biomarkers. An American Thoracic Society/National Heart, Lung, and Blood Institute Research Statement. Am J Respir Crit Care Med 2018; 198: 116-136.

13) Song C, He L, Zhang J, Ma H, Yuan X, Hu G, Tao L, Zhang J, Meng J. Fluorofenidone attenuates pulmonary inflammation and fibrosis via inhibiting the activation of NALP3 inflammasome and IL-1β/IL-1R1/MyD88/NF-κB pathway. J Cell Mol Med 2016; 20: 2064-2077.

14) De Lauretis A, Sestini P, Pantelidis P, Hoyles R, Hansell DM, Goh NS, Zappala CJ, Visca D, Maher TM, Denton CP, Ong VH, Abraham DJ, Kelleher P, Hector L, Wells AU, Renzoni EA. Serum interleukin 6 is predictive of early functional decline and mortality in interstitial lung disease associated with systemic sclerosis. J Rheumatol 2013; 40: 435-446.

15) Rosas IO, Richards TJ, Konishi K, Zhang Y, Gibson K, Lokshin AE, Lindell KO, Cisneros J, Macdonald SD, Pardo A, Sciurba F, Dauber J, Selman M, Gochuico BR, Kaminski N. MMP-1 and MMP-7 as potential peripheral blood biomarkers in idiopathic pulmonary fibrosis. PLoS Med 2008; 5: 93-97.

16) Ancochea J, Girón RM, López-Botet M. Producción de factor de necrosis tumoral alfa e interleucina 6 por los macrófagos alveolares de pacientes con artritis reumatoide y enfermedad pulmonar intersticial [Production of tumor necrosis factor alpha and interleukin-6 by alveolar macrophages from patients with rheumatoid arthritis and interstitial pulmonary disease]. Arch Bronconeumol 1997; 33: 335-340.

17) Hirani N, MacKinnon AC, Nicol L, Ford P, Schambye H, Pedersen A, Nilsson UJ, Leffler H, Sethi T, Tantawi S, Gravelle L, Slack RJ, Mills R, Karmakar U, Humphries D, Zetterberg F, Keeling L, Paul L, Molyneaux PL, Li F, Funston W, Forrest IA, Simpson AJ, Gibbons MA, Maher TM. Target inhibition of galectin-3 by inhaled TD139 in patients with idiopathic pulmonary fibrosis. Eur Respir J 2021; 57: 1-7.

18) He Q, Tang Y, Huang J, Rao Y, Lu Y. The value of KL-6 in the diagnosis and assessment of interstitial lung disease. Am J Transl Res 2021; 13: 9216-9223.

19) Xia F, Liu H, Zhang H, Ping N, Wang P, Jin Z, Zhu J, Changju Qu. Multiple blood parameters may serve as a warning to immunochemotherapy-related interstitial lung disease in B-cell lymphoma. Ann Palliat Med 2021; 10: 9660-9668.

20) Ruta VM, Man AM, Alexescu TG, Motoc NS, Tarmure S, Ungur RA, Todea DA, Coste SC, Valean D, Pop MC. Neutrophil-To-Lymphocyte Ratio and Systemic Immune-Inflammation Index-Biomarkers in Interstitial Lung Disease. Medicina (Kaunas) 2020; 56: 1-9.

21) Saku A, Fujisawa T, Nishimoto K, Yoshimura K, Hozumi H, Karayama M, Suzuki Y, Furuhashi K, Enomoto N, Nakamura Y, Inui N, Suda T. Prognostic significance of peripheral blood monocyte and neutrophil counts in rheumatoid arthritis-associated interstitial lung disease. Respir Med 2021; 182: 1-5.

22) Lou Y, Zheng Y, Fan B, Zhang L, Zhu F, Wang X, Chen Z, Tan X. Serum S100A12 levels are correlated with clinical severity in patients with dermatomyositis-associated interstitial lung disease. J Int Med Res 2020; 48: 1-8.

23) Gupta S, Padhan P, Subhankar S, Singh P. Cardiovascular complications in patients with interstitial lung disease and their correlation with 6-minute walk test and spirometry: A single-center study. J Family Med Prim Care 2021; 10: 3330-3335.

24) Kaya F, Özgül E, Balcı A. Quantitative and visual analysis of idiopathic pulmonary fibrosis with different methods: the relationship between clinical correlation and mortality risk model. Eur Rev Med Pharmacol Sci 2021; 25: 3254-3263.

25) Warrick JH, Bhalla M, Schabel SI, Silver RM. High resolution computed tomography in early scleroderma lung disease. J Rheumatol 1991; 18: 1520-1528.

26) ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002; 166: 111-117.

27) Cheresh P, Kim SJ, Tulasiram S, Kamp DW. Oxidative stress and pulmonary fibrosis. Biochim Biophys Acta 2013; 1832: 1028-1040.

28) Jouneau S, Crestani B, Thibault R, Lederlin M, Vernhet L, Valenzuela C, Wijsenbeek M, Kreuter M, Stansen W, Quaresma M, Cottin V. Analysis of body mass index, weight loss and progression of idiopathic pulmonary fibrosis. Respir Res 2020; 21: 1-11.

29) Kulkarni T, Yuan K, Tran-Nguyen TK, Kim YI, de Andrade JA, Luckhardt T, Valentine VG, Kass DJ, Duncan SR. Decrements of body mass index are associated with poor outcomes of idiopathic pulmonary fibrosis patients. PLoS One 2019; 14: 1-7.

30) Satoh H, Kurishima K, Ishikawa H, Ohtsuka M. Increased levels of KL-6 and subsequent mortality in patients with interstitial lung diseases. J Intern Med 2006; 260: 429-434.

31) Bergantini L, d'Alessandro M, Vietri L, Rana GD, Cameli P, Acerra S, Sestini P, Bargagli E. Utility of serological biomarker' panels for diagnostic accuracy of interstitial lung diseases. Immunol Res 2020; 68: 414-421.

32) Zhang Y, Kaminski N. Biomarkers in idiopathic pulmonary fibrosis. Curr Opin Pulm Med 2012; 18: 441-446.

33) Enomoto Y, Matsushima S, Shibata K, Aoshima Y, Yagi H, Meguro S, Kawasaki H, Kosugi I, Fujisawa T, Enomoto N, Inui N, Nakamura Y, Suda T, Iwashita T. LTBP2 is secreted from lung myofibroblasts and is a potential biomarker for idiopathic pulmonary fibrosis. Clin Sci (Lond) 2018; 132: 1565-1580.

34) Aoshima Y, Enomoto Y, Muto S, Meguro S, Kawasaki H, Kosugi I, Fujisawa T, Enomoto N, Inui N, Nakamura Y, Suda T, Iwashita T. Gremlin-1 for the Differential Diagnosis of Idiopathic Pulmonary Fibrosis Versus Other Interstitial Lung Diseases: A Clinical and Pathophysiological Analysis. Lung 2021; 199: 289-298.

35) Hecker L. Mechanisms and consequences of oxidative stress in lung disease: therapeutic implications for an aging populace. Am J Physiol Lung Cell Mol Physiol 2018; 314: 642-653.

36) Singh VP, Bali A, Singh N, Jaggi AS. Advanced Glycation End Products and Diabetic Complications. Korean J Physiol Pharmacol 2014; 18: 1-14.

37) Du S, Ai J, Zeng X, Wan J, Wu X, He J. Plasma level of advanced oxidation protein products as a novel biomarker of acute lung injury following cardiac surgery. Springerplus 2016; 5: 1-7.

38) Ruta VM, Man AM, Alexescu TG, Motoc NS, Tarmure S, Ungur RA, Todea DA, Coste SC, Valean D, Pop MC. Neutrophil-To-Lymphocyte Ratio and Systemic Immune-Inflammation Index-Biomarkers in Interstitial Lung Disease. Medicina (Kaunas) 2020; 56: 1-9.

39) Peterson MW, Monick M, Hunninghake GW. Prognostic role of eosinophils in pulmonary fibrosis. Chest 1987; 92: 51-56.

40) Koma Y, Onishi A, Matsuoka H, Oda N, Yokota N, Matsumoto Y, Koyama M, Okada N, Nakashima N, Masuya D, Yoshimatsu H, Suzuki Y. Increased red blood cell distribution width associates with cancer stage and prognosis in patients with lung cancer. PLoS One 2013; 8: 1-7.