KL-6, ET-1 and S100A9 levels in patients with idiopathic pulmonary fibrosis and obstructive sleep apnea

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Abstract. Obstructive sleep apnea (OSA) and idiopathic pulmonary fibrosis (IPF) frequently coexist. Elevated levels of Krebs von den Lungen-6 (KL-6), endothelin-1 (ET-1) and S100 calcium-binding protein A9 (S100A9) have been observed in patients with IPF, suggesting their potential role as biomarkers for lung fibrosis. The aim of the present study was to measure the levels of KL-6, ET-1 and S100A9 in patients with IPF-OSA and to test the potential of these biomarkers as a characteristic OSA signature with diagnostic and prognostic potential for IPF. A total of 55 subjects with newly-diagnosed IPF participated in the present cross-sectional study. In addition to performing overnight attended polysomnography and pulmonary function tests, serum and bronchoalveolar lavage (BAL) levels of KL-6, along with serum levels of ET-1 and S100A9, were also assessed. A total of 15 patients with IPF and 40 patients with IPF-OSA were included. Age, sex, comorbidities and pulmonary function tests did not differ between the groups. Although there was no significant difference between groups in the levels of KL-6, ET-1 and S100A9 ($P>0.05$), the serum ET-1 levels tended to be elevated in patients with OSA-IPF compared with patients with IPF (1.78 vs. 1.07 pg/ml; $P=0.06$). Additionally, a significant association was observed between serum KL-6 levels and the severity of IPF, and also between BAL KL-6 levels and nocturnal mean SaO_2 levels, even after taking into account factors such as obesity and smoking. S100A9 levels were associated with the oxygen desaturation index, even after adjustments for obesity, smoking and the gender-age-physiology index, only in the IPF-OSA group. Conclusively, the present findings suggested significant associations between serum ET-1, S100A9 and BAL KL-6 levels and specific OSA severity parameters in the IPF-OSA group. This evidence suggested that these molecules could serve as biomarkers for the identification of patients with IPF-OSA, offering a distinct OSA signature that has diagnostic and potential treatment value. Larger studies are crucial to substantiate the present findings and reinforce this hypothesis.

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Introduction

Idiopathic Pulmonary Fibrosis (IPF) is a distinct type of interstitial lung disease (ILD) with an unknown etiology, characterized by low survival rates, predominantly affecting older individuals (1,2). Despite IPF primarily affecting a single organ, there are numerous comorbidities that can affect the prognosis and alter the natural course of the disease (3,4). In fact, sleep-related disorders, including Obstructive Sleep Apnea (OSA), have been acknowledged as a significant comorbidity with a high prevalence in individuals diagnosed with IPF (5-8).

OSA, a disorder with high prevalence, is frequently overlooked and has significant associations with detrimental consequences, notably cardiovascular disease and sudden death (9-11). Irrespective of the severity of IPF, the presence of OSA has been found to be correlated with a poor outcome (12,13). Furthermore, severe OSA in IPF patients has been strongly associated with the presence of cardiovascular diseases and with increased systemic oxidative stress and blood biomarkers associated with lung fibrosis (14,15).

Established airway or blood biomarkers in lung fibrosis are still missing, despite recent advances in the pathobiology of the disease development (16,17). However, elevated levels of Krebs von den Lungen-6 (KL-6), Endothelin-1 (ET-1), and S100 calcium-binding protein A9 (S100A9) have been observed in patients with IPF, indicating that they could serve as biomarkers for diagnosis and prognosis (18,19). Importantly, a comprehensive assessment of these biomarkers in patients with OSA and IPF has not yet been conducted. Therefore, the aim of this study was to investigate (1) potential differences in serum and bronchoalveolar lavage (BAL) KL-6 levels, and serum ET-1 and S100A9 levels in IPF-OSA patients, and (2) test the hypothesis that assessment of these biomarkers provides a characteristic OSA signature, potentially valuable for diagnostic screening and treatment monitoring.

Materials and methods

Study design. We performed a cross-sectional study on newly diagnosed patients with IPF, who were admitted to the Sleep Disorders Center at the Medical School of the University of Crete, Greece, for the evaluation of OSA between December 2013 and December 2017. In order to be included in the study, patients had to have histologically confirmed IPF (usual interstitial pneumonia) through surgical lung biopsy. Alternatively, if a surgical biopsy was not performed, they were eligible if they met the diagnostic criteria for IPF outlined by the American Thoracic Society, European Respiratory Society, and American College of Chest Physicians (2). Patients were deemed eligible for inclusion in the study if they exhibited clinical stability for a period of no less than 4 weeks prior to enrollment and possessed an educational background surpassing elementary school. The exclusion criteria were: refusal to participate, previous OSA diagnosis, history of thoracic surgery or surgery in the upper respiratory tract, Central Sleep Apnea Syndromes, Congestive Heart Failure (NYHA III-IV), a history of life-threatening arrhythmias, severe cardiomyopathy, significant chronic kidney disease, untreated hypothyroidism, family or personal history of mental illness, drug or alcohol abuse, sedative use, severe cognitive impairment (MMSE score ≤9), concurrent oncological diseases, history of narcolepsy or restless legs syndrome.

All subjects provided written informed consent and ethical approval was provided by the University Hospital Ethics Committee of the University Hospital of Heraklion (IRB number: 1045 and 17030).

Initial visit-data collection. A detailed evaluation was conducted on all patients, encompassing various aspects such as age, body mass index (BMI) measurement, comprehensive medical history with a focus on sleep-related symptoms, associated conditions, comorbidities, smoking history, and alcohol intake. In addition, we performed pulmonary function tests (PFTs), overnight attended polysomnography (PSG) and measurement of arterial blood gases (ABGs).

Pulmonary function tests. All patients underwent PFTs and recording of O_2 saturation (SpO2) by noninvasive pulse oximetry. We followed standardized procedures to conduct spirometry and assess the carbon monoxide diffusing capacity of the lung (DLco) (20,21). Spirometry was performed with the patient in the upright and supine position. Furthermore, we utilized the gender-age-physiology (GAP) index, a comprehensive prognostic staging system, to summarize the clinical-functional severity in patients with IPF. This index incorporates various clinical and physiological variables such as gender, age, forced vital capacity (FVC), and DLco (22). The patients were categorized into three stages based on the GAP index: stage 1 included 24 patients with a GAP index ranging from 0 to 3, stage 2 consisted of 15 patients with a GAP index of 4 to 5, and stage 3 comprised 6 patients with a GAP index greater than 5.

Questionnaires. All patients filled out the Epworth Sleepiness Scale (ESS), Beck Depression scale (BDS) and quality of life questionnaire (Short-Form-36, SF-36).

Epworth sleepiness scale (ESS). Currently, the ESS is the most commonly utilized subjective test for assessing daytime sleepiness in clinical settings. This self-administered questionnaire is straightforward and consists of eight items. It measures the risk of falling asleep in specific everyday situations. A score of 10 or below is considered to be within the normal range. As the score increases (ranging from 10 to 24), the level of reported daytime sleepiness also increases (23).

Beck depression inventory (BDI). The 21-item questionnaire is a widely recognized and extensively validated self-report measure of depressive symptoms. The BDI assesses the intensity of depressive symptoms experienced in the week prior. The respondent rates each item by selecting one or more options, ranging from 0 (no symptoms) to 3 (most severe level). Scores can range from 0 to 63, representing the total of the highest level endorsed on each item. Any score below 10 is considered to be within the normal range (24).

Short-Form 36 Health Survey: This questionnaire, consisting of 36 items, is a reliable and validated tool for assessing the general health and quality of life. The SF-36 health survey consists of eight domains, each scored on a scale from 0 (worst) to 100 (best). The SF-36 scales are classified into two dimensions, namely physical health and mental health. The scale for the score is 0 to 100, with 100 representing the highest quality of life and 0 representing the lowest (25).

Polysomnography (PSG). Each patient underwent a single-night full diagnostic Polysomnography (PSG) study (Alice 5, Diagnostics System, Respironics, USA) following standard procedures, with monitoring of the electroencephalogram (EEG) (using three EEG derivations, frontal, central, and occipital), electro-oculogram, electromyogram, flow (by oronasal thermistor and nasal air pressure transducer), thoracic and abdominal respiratory effort (by respiratory inductance plethysmography), pulse oximetry (SpO2), and body position monitoring. Snoring was recorded by a microphone placed on the anterior neck. The definition of apnea and hypopnea followed the American Academy of Sleep Medicine (AASM) standard criteria (26). The apnea-hypopnea index (AHI), calculated as the number of apnea and hypopnea events per hour of sleep, was used to diagnose OSA and assess its severity. OSA was considered mild if the AHI was ≥5 per h but <15 per h, as moderate if AHI was ≥15 per h but <30 per h, and as severe if AHI was ≥30 per h.

Biomarker measurements. Once overnight polysomnography was completed, blood samples were collected in the morning after fasting overnight. Bronchoalveolar lavage (BAL) was obtained from patients with a flexible bronchoscope wedged into a subsegmental bronchus of a predetermined region of interest based on radiographical findings. A total of 120 ml of normal saline were instilled in 60-ml aliquots, retrieved by low suction. The BAL fractions were pooled and split equally into two samples. Bronchoalveolar lavage fluid (BALF) was passed through a Millipore filter to isolate cells in suspension from debris and mucus. To pellet cells, samples were centrifuged at 1,500 rpm for five minutes at room temperature (RT) and BAL supernatant was stored in aliquots at -80˚C. KL-6 levels were measured in the supernatant. Serum and BAL KL-6 levels were measured using Nanopia KL-6 assay

(Sekusui Diagnostics GMBH) with OLYMPUS AU640, and were expressed as Units/ml according to the manufacturer's instructions. The KL-6 level in healthy individuals has a reference range of 105.3-401.2 U/ml according to manufacturer's instructions. ET-1 serum concentration was measured with Endothelin-1 Quantikine ELISA (R&D Systems, DET100) and expressed as pg/ml, according to manufacturer's instructions. The mean ± standard deviation (SD) ET-1 levels in healthy volunteers are 1.24±0.35 pg/ml with a range of 0.47-2 pg/ml according to the manufacturer. S100A9 serum concentration was measured with human S100A9 DuoSet ELISA (R&D Systems, DY5578) and were expressed as μg/ml according to manufacturer's instructions.

Statistical analysis. Results are presented as mean ± SD for continuous variables if normally distributed, and as median (25-75th percentile) if not. Absolute numbers (or percentages) are used to represent qualitative variables. To compare groups, we used a two-tailed unpaired t-test for independent samples (when data was normally distributed) or a Mann-Whitney U test (when data was not normally distributed) for continuous variables. For categorical variables, Fisher's exact test or the χ^2 test was used. To determine the relationship between different parameters and inflammatory biomarker levels, we used the Spearman's correlation test (non-normally distributed data) to calculate correlation coefficients for all the independent predictors. We included various clinically relevant factors as independent variables, such as age, gender, BMI, smoking history, comorbidities, AHI, oxygen desaturation index (ODI), average and minimum SpO2 levels during sleep, duration of time with SpO2 below 90%, arterial blood gas (ABG) measurements, and spirometry data. In addition, we employed multivariate linear regression analysis to investigate the potential relationship between biomarker levels and indices of respiratory function and severity of OSA. Potential explanatory variables, such as age, gender, BMI, GAP index, PFTs, indices of OSA severity, smoking status, and co-morbidities, were taken into account when adjusting all models. A P-value less than 0.05 was deemed to be statistically significant. Data were analyzed using PAWP 17.0 software (SPSS Inc, Chicago, IL).

Results

Patient characteristics and polysomnographic findings. Fifty-five subjects (46 males, 9 females) were included in the study (Fig. 1). At the time of the study, all patients were treatment naïve and did not receive corticosteroids or require oxygen supplementation. Using AHI ≥15 for OSA diagnosis resulted in: 15 patients with IPF (27%), and 40 IPF-OSA (73%). Table I summarizes the clinical variables collected for the two groups. Age, gender, BMI, comorbidities, smoking status and PFTs were not different between the two groups (P>0.05). As expected, AHI, ODI, mean SaO_2, min SaO_2, and Total Sleep Time with oxygen saturation below 90% (TST 90) were worse in the IPF-OSA group (Table II). Concerning sleepiness, depressive symptoms, and quality of life, patients with IPF-OSA showed more severe functional impairments, which reached statistical significance only for Physical Component Summary (PCS) of the SF-36 (56 vs. 67, P=0.03).

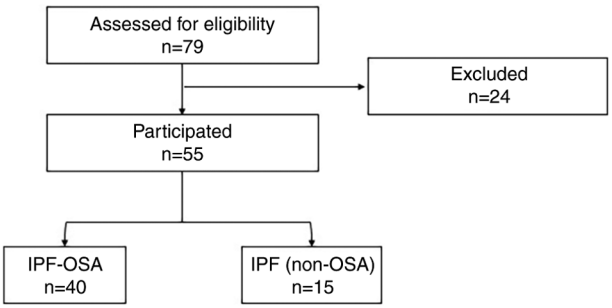

Figure 1. Flow diagram showing how the final sample was obtained. IPF, idiopathic pulmonary fibrosis; OSA, obstructive sleep apnea.

Evaluation of KL-6, ET-1 and S100A9 levels. Table III shows the results of the measurement of the three studied molecules; KL-6, ET-1 and S100A9, in the whole IPF population and per group. Serum KL-6 levels were found increased in comparison with BAL KL-6 levels in all IPF patients, while no difference was observed between patients with IPF and patients with IPF and OSA (P>0.05), either in serum or in BAL (Table III; Fig. 2).

Regarding ET-1, increased serum levels were detected in the IPF-OSA group, compared with IPF patients without OSA, although this result was marginally statistically significant (P=0.06) (Table III; Fig. 3). Interestingly, subgroup analysis based on OSA severity, revealed further increase of ET-1 in patients with severe OSA (AHI ≥30) compared to IPF-non-OSA group (1.74 vs. 1.07, P=0.07).

S100A9 serum levels were also evaluated, but no significant difference was found between the two studied groups of patients (Table III; Fig. 4).

Correlation analysis. In the whole studied population, significant correlations were found between serum KL-6 levels and the severity of IPF, assessed by GAP index (r=0.507, P=0.001), TLC (%) (r=-0.43, P=0.008), DLCO (%) (r=-0.46, P=0.005), and KCO (%) (r=-0.38, P=0.02) (Table SI). Serum levels of KL-6 were still correlated with GAP index independently of obesity, smoking, or indices of OSA severity (β=321.7, P=0.001). Furthermore, BAL KL-6 levels were correlated with nocturnal mean SaO_2 (r=-0.49, P=0.028), even after adjustment for obesity, smoking and GAP index (β=-25.273, P=0.04) and S100A9 levels (r=0.509, P=0.019). Correlations were also found between ET-1 and GAP index (r=0.365, P=0.006), DLCO (%) (r=-0.53, P<0.001), KCO (%) (r=-0.54, P<0.001) and TST90 (r=0.32, P=0.021) (Table SII). However, these correlations disappeared after adjustment for obesity and smoking.

Furthermore, we analyzed the correlation between the indices of OSA severity, arterial blood gases, pulmonary function, and the levels of these markers separately in the two groups, in order to evaluate a possible association of these molecules in IPF-OSA patients. Interestingly, serum KL-6 was correlated with nocturnal mean SaO_2 only in the IPF group (r=0.73, P=0.011), and BAL KL-6 correlated with AHI (r=0.55, P=0.04) and with nocturnal mean SaO_2 (r=-0.66, P=0.01) only in the IPF-OSA group. All these correlations persisted, although with a marginally statistical significance, after adjustments for obesity, smoking and GAP index

Table I. Baseline demographics, spirometric measurements and ABG analysis results of the included patients.

Characteristics	All patients (n=55)	IPF (n=15)	IPF-OSA (n=40)	P-value
Age, years	73.4±5.7	73.9±5.6	73.2±5.9	0.69
Male, n (%)	46 (84)	13 (87)	33 (83)	0.71[a]
BMI, kg/m^2	30.4±4.1	29.6±3.6	30.8±4.3	0.36
Neck circumference, cm	41.2±2.9	41.6±2.4	41.1±3.0	0.62
Waist circumference, cm	110.5±10.2	106.9±9.4	110.7±10.4	0.13
Hip circumference, cm	106.9±8.6	105.6±6.9	107.4±9.1	0.51
Smoking status, n (%)				0.33[b]
Current smokers	3 (6)	1 (7)	2 (5)	
Ex-smokers	37 (67)	8 (53)	29 (73)	
Comorbidities, n (%)				
Diabetes mellitus	19 (35)	5 (33)	14 (35)	0.99[b]
Hypertension	30 (55)	8 (53)	22 (55)	0.91[a]
Dyslipidaemia	20 (36)	6 (40)	14 (35)	0.76[b]
Ischemic heart disease	10(18)	2 (13)	8 (20)	0.71[b]
Atrial fibrillation	4 (7)	0 (0)	4 (10)	0.57[b]
Compensated heart failure	2 (4)	0 (0)	2 (5)	0.99[b]
Hypothyroidism	7 (13)	3 (20)	4 (10)	0.38[b]
COPD	11 (20)	3 (20)	8 (20)	0.99[b]
GAP index, n (%)				0.37[b]
Stage I	16 (29)	3 (20)	13 (32)	
Stage II	32 (58)	10 (67)	22 (55)	
Stage III	7 (13)	2 (13)	5 (13)	
PFTs				
FVC, % predicted	75.4±17.0	74.9±18.1	75.6±16.9	0.89
FEV_1/FVC	82.0±6.0	83.4±6.1	81.5±6.0	0.31
TLC, % predicted	69.8±15.1	64.6±16.9	71.8±14.1	0.12
DLCO, % predicted	50.7±16.7	50.7±16.6	50.7±17.0	0.99
KCO, % predicted	87.0±22.9	88.8±22.0	86.4±23.5	0.73
ABGs				
pH	7.41±0.02	7.42±0.02	7.41±0.02	0.13
$pPCO_2$, mmHg	39.4±3.7	38.5±3.1	39.7±3.9	0.32
pPO_2, mmHg	71.2±8.2	70.2±8.7	71.6±8.2	0.59
HCO_3^-, mmol/l	25.1±2.0	24.9±1.5	25.2±2.2	0.73

A two-tailed t-test for independent samples (for normally distributed data) was used for continuous variables, and the [a]χ^2 test or [b]Fisher's exact test was used for categorical variables. P<0.05 was considered to indicate a statistically significant difference. Data are presented as the mean ± SD, unless otherwise indicated. Smoking status was defined as current smokers and ex-smokers, while the rest of the patients were never smokers. COPD, chronic obstructive pulmonary disease; GAP, gender-age-physiology; PFT, pulmonary function test; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 sec; TLC, total lung capacity; DLCO, predicted diffusing capacity of the lung for carbon monoxide; KCO, carbon monoxide transfer coefficient; ABG, arterial blood gas; IPF, idiopathic pulmonary fibrosis; OSA, obstructive sleep apnea.

(β=221.718, P=0.05, β=3.566, P=0.04 and β=-29.969, P=0.08 respectively).

ET-1 was correlated with TST90 (Table SII) in IPF-OSA group (r=0.32, P=0.04), but this correlation disappeared after adjustments for obesity, smoking and GAP index. In the IPF-OSA group S100A9 levels were also correlated with indices of OSA severity (Table SIII), including TST90 (r=0.34, P=0.03) and with a borderline significance after adjustments for obesity, smoking and GAP index (β=0.015, P=0.06), ODI (r=0.36, P=0.023) which persisted after adjustments for obesity, smoking and GAP index (β=0.013, P=0.02), and nocturnal mean SaO_2 (r=-0.32, P=0.04), but this correlation disappeared after adjustments for obesity, smoking and GAP index.

Discussion

The prevalence of OSA exhibited a persistent upward trend, in patients with IPF, with reported prevalence ranging from

Table II. Baseline PSG data and questionnaires scores of the final sample.

Variables	All patients (n=55)	IPF (n=15)	IPF-OSA (n=40)	P-value
Diagnostic PSG				
TRT, min	435±40	425±46	438±37	0.28
TST, min	235±57	216±67	242±52	0.13
SE, %	54±12	51±14	55±11	0.22
WASO, min	153±52	155±64	153±48	0.92
NREM, %TST	91±3	91±3	91±3	0.99
SWS, %TST	7 (6-10)	7 (7-10)	7 (6-9)	0.49
REM, %TST	9±3	9±3	9±3	0.99
AHI, events/h	22 (13-40)	8 (4-12)	26 (5-12)	<0.01
REM AHI, events/h	31 (13-48)	6.5 (1.0-9.25)	39 (30-58)	<0.01
AI, /h	45±13	37±12	49±11	<0.01
ODI, events/h	26 (18-40)	9 (4-14)	30 (23-54)	<0.01
Mean SaO_2, %	92 (89-93)	94 (92-94)	91 (86, 92)	<0.01
Minimum SaO_2, %	81 (78-84)	86 (85-89)	80 (74-82)	<0.01
TST90, min	40 (18-141)	5 (0-10)	95 (33-195)	<0.01
Questionnaire scores				
ESS	7±5	7±5	8±5	0.53
ESS ≥10, n (%)	20 (36)	5 (33)	15 (38)	0.54[a]
BDS	11±5	10±5	11±6	0.51
BDS ≥10, n (%)	31 (56)	6 (40)	25 (63)	0.13[b]
SF-36				
PF	62±22	70±16	39±23	0.07
RP	56±29	67±29	52±28	0.08
BP	76±22	77±18	75±23	0.78
GH	54±17	60±16	51±17	0.11
PCS	59±17	67±16	56±17	0.03
VT	55±16	57±16	54±18	0.56
SF	78±19	80±20	77±20	0.68
RE	70±26	74±28	68±26	0.45
MH	64±15	67±9	63±17	0.46
MCS	64±15	70±16	61±15	0.08

A two-tailed t-test for independent samples (for normally distributed data) or a Mann-Whitney U test (for non-normally distributed data) was used for continuous variables, while [a]Fisher's exact test or the [b]χ^2 test was used for categorical variables. P<0.05 was considered to indicate a statistically significant difference. Data are presented as the mean ± SD or median (interquartile range), unless otherwise indicated. PSG, polysomnography; TRT, total recording time; TST, total sleep time; SE, sleep efficiency; WASO, wake after sleep onset time; NREM, non-rapid eye movement; SWS, slow wave sleep; REM, rapid eye movement; AI, arousal index; AHI, apnoea-hypopnoea index; ODI, oxygen desaturation index; TST90, total sleep time with oxygen saturation <90%; ESS, Epworth Sleepiness Scale; BDS, Beck Depression scale; SF-36, Short-Form-36; PF, physical functioning; RP, role physical; BP, bodily pain; GH, general health; PCS, physical component; VT, vitality; SF, social functioning; RE, role emotional; MH, mental health; MCS, mental component; IPF, idiopathic pulmonary fibrosis; OSA, obstructive sleep apnea.

10 to 88% in several cohorts (6-8,13,27-29). The underlying pathophysiologic mechanisms of this relationship are still not fully understood. However, there is data implicating intermittent hypoxia and aging-related mechanisms in OSA, such as oxidative stress and short telomere length, in the pathogenesis or disease progression of pulmonary fibrosis (15,27). On the other hand, it could be expected that restrictive lung diseases, including IPF, are characterized by reduced lung volumes which could induce upper airway instability and promote OSA (30).

Our study investigated the levels of three molecules that have been associated with lung fibrosis in order to evaluate potential differences between IPF patients with and without OSA, Particularly, we assessed the levels of ET-1 in serum, as well as the levels of KL-6 in serum and BAL, and S100A9 in serum, in a group of consecutive IPF patients who were suspected to have OSA. Only newly diagnosed and treatment-naïve IPF patients were included in the current study. Therefore, we evaluated patients upon IPF diagnosis and investigated the presence of comorbid OSA. We compared patients

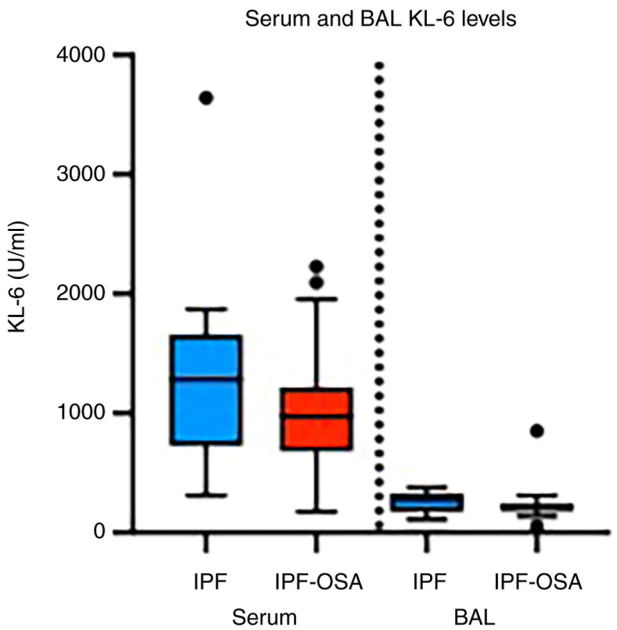

Figure 2. Serum and BAL KL-6 levels (U/ml) in patients with IPF compared with patients with IPF-OSA. Data are presented as the median (interquartile range). The Mann-Whitney U test was used for the comparison between the groups. P<0.05 was considered to indicate a statistically significant difference. BAL, bronchoalveolar lavage; IPF, idiopathic pulmonary fibrosis; KL-6, Krebs von den Lungen-6; OSA, obstructive sleep apnea.

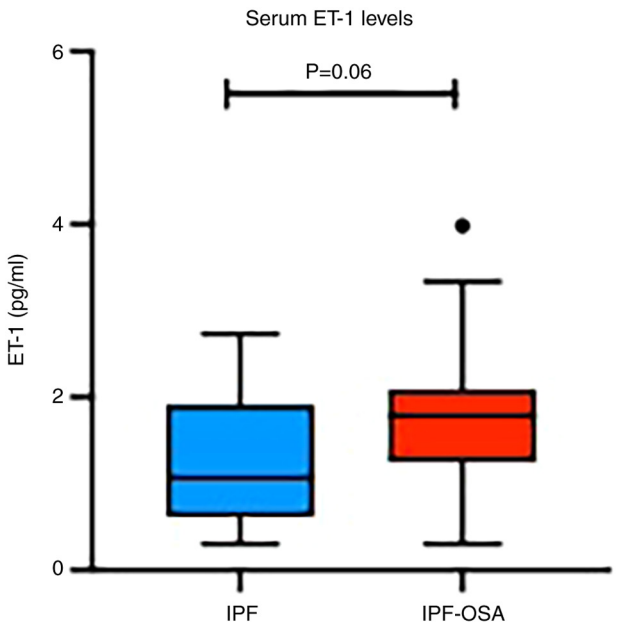

Figure 3. Serum ET-1 levels (pg/ml) in patients with IPF compared with patients with IPF-OSA. Data are presented as the median (interquartile range). The Mann-Whitney U test was used for the comparison between the groups. P<0.05 was considered to indicate a statistically significant difference. ET-1, endothelin-1; IPF, idiopathic pulmonary fibrosis; OSA, obstructive sleep apnea.

with both IPF and OSA vs. IPF without OSA, although it remains indeterminable whether OSA or IPF developed first in these patients. Our findings showed an increase in serum ET-1 in IPF-OSA group, when compared with IPF patients without OSA, while it was correlated statistically significant with OSA severity parameters. In the whole studied population, ET-1 levels were significantly correlated with GAP index and pulmonary function tests. Moreover, independent associations of serum KL-6 levels and IPF severity index were detected. Significant associations were also noted in BAL KL-6 and serum S100A9 levels with specific OSA severity parameters in IPF-OSA subgroup.

ET-1 is a peptide hormone primarily synthesized by the vascular endothelium, initially described for its role in vasoconstriction, although it has been also involved in pulmonary fibrosis pathogenesis, as a mediator of transforming growth factor (TGF)-b pathway and enhancer of fibroblasts differentiation and collagen production (19,31,32). Current literature has described an association between serum ET-1 levels and IPF severity, suggesting a role as a potential disease biomarker (33,34). In our study, ET-1 levels were increased in the IPF-OSA compared to IPF group (1.78 vs. 1.07), and this difference seemed to be driven by the OSA severity. Furthermore, ET-1 levels were associated with PFTs and indices of OSA severity, although these correlations disappeared after adjustment for obesity and smoking. As ET-1 levels in OSA patients are associated with increased cardiovascular risk (35), larger and prospective studies are needed to clarify the role of ET-1 in IPF patients with OSA.

KL-6, a mucin-like integral membrane glycoprotein, localized to type II alveolar epithelial cells and bronchial epithelial cells, has been found elevated in the blood of patients with lung injury, while its levels have been also associated with severity and prognosis in patients with pulmonary fibrosis (36-38). In our study, serum KL-6 levels were found increased in comparison with BAL levels, and they were correlated with IPF severity, in accordance with current literature (38). Importantly, our analysis in IPF-OSA patients revealed that BAL KL-6 levels were correlated with indexes of OSA severity, independently of obesity or smoking, implying a possible further epithelial injury in IPF-OSA patients. While the exact role of KL-6 as a biomarker for lung injury in OSA remains unclear, a previous study with a small sample size indicated that some patients with OSA had elevated circulating levels of KL-6, proposing the possibility of subclinical lung injury in OSA (39). Despite the potential influence of comorbid obesity and smoking exposure on KL-6 levels and lung diffusion capacity, our study demonstrated that even after accounting for these factors, the correlation between serum KL-6 and pulmonary function impairment, as well as the relationship between BAL KL-6 and indices of OSA severity, remained statistically significant (30,31).

S100A9, additionally known as myeloid-related protein 14 or calgranulin B, belongs to the family of calcium-binding proteins S100. It is mostly expressed in neutrophils, but also in endothelial cells and macrophages, exerting immunomodulatory and profibrotic properties (40). Previous studies have suggested a role of S100A9 for diagnosis and prognosis in interstitial lung diseases (40-42). In our study, serum S100A9 levels were correlated significantly with indices of OSA severity in patients with IPF and OSA, even after adjustments for obesity, smoking and GAP index. As far as we are concerned, our study is the first one evaluating the levels of S100A9 in serum

Table III. Levels of serum KL-6, BAL KL-6, ET-1 and S100A9 in the entire cohort and in the two groups.

Variables	All patients (n=55)	IPF (n=15)	IPF-OSA (n=40)	P-value
Serum KL-6 (U/ml)	1087 (726-1407)	1280 (729-1659)	975 (682-1217)	0.12
BAL KL-6 (U/ml)	205 (183-294)	271 (175-330)	205 (183-245)	0.59
ET-1 (pg/ml)	1.74 (0.99-2.03)	1.07 (0.63-1.90)	1.78 (1.00-1.98)	0.06
S100A9 (μg/ml)	0.89 (0.05-0.17)	0.14 (0.05-0.72)	0.08 (0.05-0.15)	0.23

Data are presented as the median (interquartile range). The Mann-Whitney U test was used for the comparison between the groups. P<0.05 was considered to indicate a statistically significant difference. KL-6, Krebs von den Lungen-6; BAL, bronchoalveolar lavage; ET-1, endothelin-1; S100A9, S100 calcium-binding protein A9; IPF, idiopathic pulmonary fibrosis; OSA, obstructive sleep apnea.

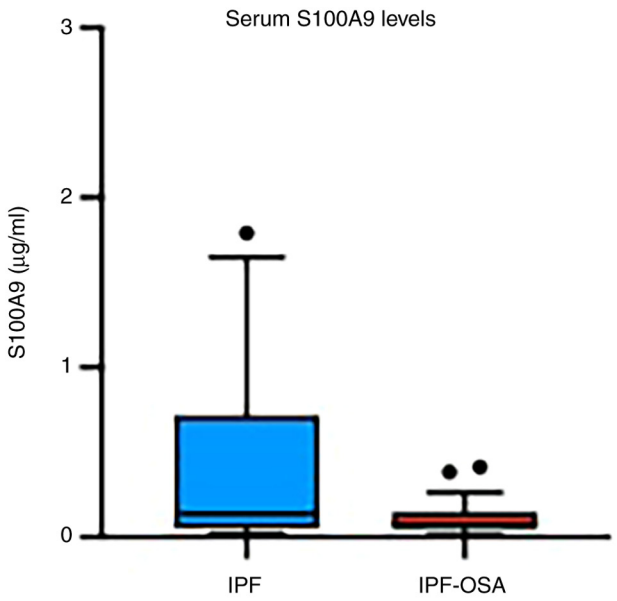

Figure 4. Serum S100A9 levels (μg/ml) in patients with IPF compared with patients with IPF-OSA. Data are presented as the median (interquartile range). The Mann-Whitney U test was used for the comparison between the groups. P<0.05 was considered to indicate a statistically significant difference. IPF, idiopathic pulmonary fibrosis; OSA, obstructive sleep apnea; S100A9, S100 calcium-binding protein A9.

of IPF patients with OSA, implying a potent association with OSA severity, which should be further investigated in larger cohorts.

The results of our study suggested that the levels of ET-1, KL-6 and S100A9, previously described as potential biomarkers for lung fibrosis, could also be associated with the presence of OSA, as well as OSA severity, in IPF patients. Our findings may provide a warning that lung injury is more prominent in patients with IPF and OSA.

Given that the clinical characteristics, progression, and mortality of IPF can be influenced by the presence of multiple comorbidities like OSA, it is imperative to prioritize the early identification and treatment of OSA alongside the treatment of IPF (4,29). Given the fact that patients commonly underestimate their symptoms and delay OSA diagnosis, it is of great importance for the clinician to seek for biomarkers that could imply a potential OSA signature in IPF patients, so further evaluation for OSA co-existence can be suggested. Moreover, a comprehensive understanding of the underlying pathogenetic mechanisms that drive the development of IPF and OSA will shed light to the identification of valuable biomarkers, which may be used as additional screening OSA tools, as well as therapeutic targets. Nonetheless, further studies are needed to uncover whether detection and intervention on specific biomarkers are promising new treatments for IPF patients with comorbid OSA.

Our study was limited by the relatively small number of participants in our cohort, which could explain the failure to detect statistically significant effects of many of the parameters measured. Therefore, further extensive research is required to validate those findings. Another limitation to be noted was that levels of the studied molecules were not assessed after treatment of OSA; thus, we could not provide data about their potent use as evaluators of treatment effect. Finally, the limitations of the cross-sectional design prevented us from drawing causal conclusions or determining the direction of the effects we observed. However, given that IPF is a relatively rare disease, the strength of our study was that we collected serum as well as BAL from well-characterized, treatment naïve IPF patients.

In conclusion, our findings showed increased serum ET-1 levels in IPF-OSA patients in comparison with IPF patients without OSA, and significant associations in serum ET-1, S100A9 and BAL KL-6 levels with specific OSA severity parameters in IPF-OSA group of patients. The data presented here may indicate that these molecules might be used as biomarkers for IPF-OSA, probably reflecting the additional lung injury of OSA in pulmonary fibrosis. Larger studies should confirm our results maybe suggesting a characteristic OSA signature with diagnostic screening value and utility in treatment monitoring.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

IB and SM contributed to the acquisition of human samples, and confirm the authenticity of the raw data. SM, EV and NT performed the bronchoscopies. ET and CK performed the experiments. IB, SM and EV reviewed the literature. IB, SM, EV and NT analyzed and interpreted the data. IB, EV, NT, SS and KMA were involved in the drafting of the manuscript. SS and KMA supervised the study and contributed to the conception and design of the study. All authors were involved in the writing of the manuscript, and all authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all patients who participated in the study. The study was approved by the Ethics Committees of the University Hospital of Heraklion (institutional review board no. 1045 and 17030; Crete, Greece).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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