

Periostin and closing volume in combined pulmonary fibrosis and emphysema

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Abstract

Background

Combined pulmonary fibrosis and emphysema (CPFE) is an entity characterized by the presence of emphysema in upper lobes and fibrosis in lower lobes. Due to the presence of the two diseases concomitantly, it may be difficult to diagnose. This study aims at a better understanding of this entity and proposes biological markers (functional and biochemical) that help in this characterization.

Methods

A prospective, observational, cross-sectional study was carried out at a reference center. Pulmonary function tests (spirometry, CO-diffusion capacity, plethysmography and single-maneuver nitrogen washout test - SBWN 2) and biochemical markers (periostin, mucin-16, PDGF-BB and TGF- β 1) were measured in groups of patients: idiopathic pulmonary fibrosis, CPFE and chronic obstructive pulmonary disease (COPD).

Results

Variables derived from SBWN 2 - closing volume (CV) / vital capacity (VC) (%) and closing capacity (CC) / total lung capacity (TLC) (%) - were found to be higher in the CPFE group compared to the Idiopathic pulmonary fibrosis (IPF) group (CV/VC%: 0.25 (0.12 – 11.01) and 13.05 (0.21 – 20.73); $p = 0.005$; CC/TLC%: 30.1 (22.4 – 37.47) and 33.69 (32.05 – 41.98); $p = 0.03$, respectively). Periostin was higher in the CPFE group than in the other groups [CPFE: 66.74 (45.21 – 90.5), IPF: 43.81 (31.97 – 56.18), COPD: 40.08 (20.66 – 50.81); $p = 0.0002$], and mucin-16 was higher in the IPF group than in the CPFE group [CPFE: 13.59 (4.16 – 28.16); IPF: 71.94 (40.46 - 164); COPD: 25.85 (9.27 – 30.29); $p = 0.02$].

Conclusions

Findings show that CPFE presents different functional and biochemical characteristics than IPF, including higher CV/VC%, CC/TLC% and periostin, whereas mucin-16 was higher in the IPF.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterized by progressive interstitial fibrosis of unknown cause.¹ Some patients, typically those with a high smoking load, develop combined pulmonary fibrosis and emphysema (CPFE).² Due to the distinct clinical, functional and radiological characteristics, CPFE has been described as a syndrome.² These distinct characteristics increase the risk of developing pulmonary hypertension and lung cancer, thus resulting in a worse prognosis.³ Therefore, it is important that differential diagnosis be performed properly and early. Nevertheless, there is no specific therapy for CPFE.²

Identifying new biological markers may facilitate the diagnosis and follow-up of patients with CPFE. Spirometry cannot effectively differentiate these patients because the coexistence of two antagonistic diseases can alter the test results and lead to underestimation of disease severity. Currently, techniques such as the nitrogen washout test have become widely used in the clinical environment.

Although the nitrogen washout test was developed approximately 60 years ago, modern software is currently used with this technique. Traditionally, the examination can be conducted for single or multiple breaths.⁴ The nitrogen washout test reveals the heterogeneity in the distribution of pulmonary ventilation and has been used to evaluate small airways in patients with obstructive diseases.^{5,6} However, no studies have evaluated the use of this method for evaluating CPFE or IPF.

While clinical-functional evaluation is important for the diagnosis and follow-up of patients with CPFE, a serum biomarker useful for diagnosis and prognosis prediction is needed. There are no accepted serum biomarkers for IPF or CPFE. Some biomarkers such as periostin have been tested and show potential.⁶⁻⁸ Other molecules that are part of the fibrogenesis cascade, such as transforming growth factor (TGF)- β_1 and platelet-derived growth factor-BB (PDGF-BB), have also been examined.⁹⁻¹¹ Additionally, mucin-16 (MUC-16 or CA-125) has been associated with increased mortality in patients with IPF and also with the development of lung cancer in patients with interstitial lung diseases.^{12,13}

Thus, this study aimed was conducted to evaluate patients with CPFE, particularly through functional assessment, and identify a possible serum biomarker for facilitating differential diagnosis.

Methods

This prospective, observational, cross-sectional study was carried out with approval from the Research Ethics Committee. All patients signed informed consent forms before participating in this study.

Patients with IPF, CPFE, and chronic obstructive pulmonary disease (COPD) were selected. The diagnosis of IPF was based on the criteria of the American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, and Latin American Thoracic Association 2011.¹⁴ All selected patients showed consistent clinical presentation and a tomographic pattern of usual interstitial pneumonia (UIP). The diagnosis of CPFE was based on the original report by Cottin et al.² as a syndrome characterized by upper lobe emphysema (well-demarcated areas of decreased attenuation with very thin wall (<1 mm) or no wall) and pulmonary fibrosis of the lower lobes (presence of features consistent with usual interstitial pneumonia) according to high resolution chest CT (HRCT). All cases of interstitial lung disease (IPF and CPFE) were confirmed by a multidisciplinary team and patients with collagen vascular disease, hypersensitivity pneumonia or other interstitial lung disease (ILD) were excluded. We chose to include only patients with a firm diagnosis of IPF and CPFE in order to avoid bias due to inclusion of patients with other type of interstitial lung diseases with different prognosis. All tests performed in the IPF and CPFE groups were obtained before patients started anti-fibrotic therapy.

Patients with chronic obstructive pulmonary disorder (COPD) were diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2017.¹⁵ COPD patients with emphysema phenotype were selected based on clinical and functional features (patients with < 2 exacerbations/year and carbon monoxide diffusion capacity <50%). Patients with other respiratory diseases such as asthma, bronchiectasis, and severe neuromuscular diseases that could alter lung function were excluded. Patients who were in infectious exacerbation at the time of selection were included only 3 months after clinical improvement.

All patients were interviewed by the examiner, after which peripheral blood samples were collected to measure periostin, mucin-16, PDGF-BB, and human TGF- β_1 and for functional evaluation (spirometry, measurement of the carbon monoxide diffusion capacity (DLco) and transfer coefficient (Kco), plethysmography of whole body, and single-breath nitrogen washout test).

All tests were performed by a single operator and with the same equipment, HD CPL (nSpire Health Inc., Longmont, CO, USA) and followed American Thoracic Society standardization.¹⁶ Knudson's equations for spirometry¹⁷ and Neder's equations^{18,19} were used to determine static pulmonary volumes, carbon monoxide diffusion capacity, and the transfer factor. The single-breath nitrogen washout test was performed (SBWN₂) using the HD PFR 3000 apparatus (nSpireHealth, Inc.). The single-breath maneuver washout was performed according to the recommendations of the American Thoracic Society/European Respiratory Society²⁰ and analysed using Buist equations.²¹

The concentration level of each biomarker in the sera of patients was determined with enzyme-linked immunosorbent assay (ELISA) kits for periostin (DuoSet ELISA; R&D Systems, Minneapolis, MN, USA; 1:50 dilution), mucin-16 (Thermo Fisher Scientific, Waltham, MA, USA; 1:2 dilution), PDGF-BB (PeproTech, Rocky Hill, NJ, USA; 1:10 dilution), and TGF- β_1 (Affymetrix, eBioscience, San Diego, CA, USA; 1:10 dilution). The sensitivities of the ELISA kits were 62.5 pg/mL for periostin, 1 U/mL for mucin-16, 32 pg/mL for PDGF-BB, and 8 pg/mL for TGF- β_1 . All ELISAs were performed according to the manufacturer's instructions. ELISAs were read in an automated microplate reader (Multiskan FC; Thermo Fisher Scientific).

Determination of statistical significance and graph preparation were conducted using GraphPad Prism version 8.0 software (GraphPad, Inc., La Jolla, CA, USA). The D'Agostino and Pearson test were used to evaluate the normal distribution of the samples. Analysis of variance, Kruskal Wallis test, and Dunn' post hoc were used to differentiate groups. Differences were considered significant when the *p* value was less than 0.05.

Results

A flow diagram design is shown in Figure 1. The ages of patients in the three groups (IPF, CPFE, and COPD) and the degree of dyspnea as assessed by the modified Medical Research Council scale did not significantly differ between groups. However, there was a significant difference in smoking load among

the three groups; COPD patients showed the highest smoking load (Table 1). Turkey's post-test analysis showed that the smoking load in the CPFE group was significantly higher than in the IPF group.

Functional analysis of the three groups revealed a significant difference in many of the parameters studied. Statistically significant values of forced expiratory volume in the first second (FEV_1), DLco, Kco, residual volume (RV)/total lung capacity (TLC), and TLC (%) were observed in the CPFE group compared to in the COPD group. However, no significant differences were observed between the IPF group and CPFE group (Table 1). The parameters evaluated by $SBWN_2$, phase III slope (SIII), and $\Delta N_{2\ 750-1250mL}$ presented values above the normal range in all groups, but the differences were not significant. However, the closing volume (CV)/vital capacity (VC) (%) and closing capacity (CC)/TLC (%) ratios were significantly higher in individuals with CPFE than in those with IPF ($p = 0.005$ and $p = 0.03$, respectively, according to Turkey's post-test). These data are presented in Table 2.

In the analysis of serum biomarkers, according to Table 3, periostin showed significantly higher values in the CPFE group than in the other two groups [CPFE: 66.74 (45.21–90.5); IPF: 43.81 (31.97–56.18); COPD: 40.08 (20.66–50.81); $p = 0.0002$]. Turkey's post-test shows that mucin-16 levels were significantly higher in the IPF group than in the CPFE group ($p = 0.02$) and were higher than in the COPD group ($p = 0.058$).

The Figures 2, 3, 4 and 5 show the comparison between all the serum biomarkers studied between the 3 groups of patients, demonstrating the statistically significant relationship with periostin and mucin-16 in their respective groups.

Discussion

Previous studies suggested that patients with CPFE form a subgroup of IPF or have a different disease,¹⁴ as they present with different clinical, radiological and functional alterations than patients with IPF.²² Isolating this group of patients can facilitate diagnosis and enable early treatment. Evaluating CPFE in a study series is difficult because many patients with various types of interstitial diseases are included, such as patients with connective tissue diseases, particularly rheumatoid arthritis²³ and non-specific fibrotic interstitial pneumonia.²⁴ The inclusion of several standards makes it difficult to assess prognosis. We included only patients with a well-established UIP pattern and identified important differences from a functional perspective and in blood biomarker profiles. In the blood samples, patients with CPFE had higher levels of periostin while patients with IPF had higher levels of mucin-16. Functionally, CV/VC (%) and CC/TLC (%) were higher in patients with CPFE than in those with IPF, suggesting increased air trapping associated with emphysema.

The smoking history was common in the three groups, while the smoking load was significantly higher in the COPD group compared to the other groups and higher in the CPFE group than in the IPF group. Patients with CPFE are typically smokers or former smokers with a history of smoking over 40 packets per year.²⁵ This suggests a minimum smoking load leading to progression of the combined disease.

However, CPFE has been reported in patients with no smoking history, suggesting that there is a genetic predisposition for the development of the syndrome.²⁴

As in previous studies, differentiating between IPF and CPFE by spirometry was difficult.^{2,26} Patients with CPFE showed flow measurements close to those with IPF and volume measurements close to those in patients with COPD. No spirometric variable could differentiate CPFE from the IPF, which agrees the results of previous studies.²² This may delay diagnosis, as spirometry is the most easily accessible pulmonary function test.²⁷

Although some studies reported lower values of DLco in patients with CPFE compared to in IPF,²⁴ including in our study and a study by Jacob et al²⁸, similar values were observed in both groups (IPF and CPFE). Interestingly, Kco was lower in the CPFE group. The coexistence of emphysema and fibrosis leads to normal or subnormal volumes and pulmonary fluxes, while DLco was substantially reduced.^{24,28}

SBWN₂ is an important tool for evaluating the homogeneity of alveolar ventilation and for the early detection of small airway changes in patients with COPD.⁵ The main parameters obtained by SBWN₂ are SIII and CV, which are directly correlated with FEV₁ and FVC in patients with COPD.⁵ SIII is characterized by changes in the N₂ concentration expired between 25% and 75% of the VC and $\Delta N_{2\ 750-1250\text{mL}}$. Both parameters reflect the distribution of ventilation, ie, whether there is homogeneity in ventilation. CV is the portion of the VC that begins after the start of airway closure (phase IV) and affects the RV. In ordinary individuals, this value is <20% of VC; higher values are observed in both obstructive and restrictive patients. The CC is the CV associated with RV.⁴

No studies comparing SBWN₂ have been conducted in patients with IPF and CPFE. Silva et al. conducted SBWN₂ in patients with systemic sclerosis and pulmonary involvement and compared them with a healthy population. They found that SIII was the most sensitive pulmonary function alteration in these patients, even when other pulmonary function tests were normal.²⁹

The SBWN₂ test showed heterogeneity in pulmonary ventilation in all three conditions (IPF, CPFE and COPD), without large differences between groups, which did not aid in the functional differentiation between IPF and CPFE. Additionally, our data suggested that patients with CPFE experienced significant air trapping, even when the lung volumes were within normal limits. Patients included in our study had evident fibrosis in CT scanning. Thus, SBWN₂ may be a sensitive test for evaluating the progression of fibrosis in patients with initial fibrosing disease.

Periostin levels were significantly higher in the serum of the CPFE group than in the other two groups. Periostin is involved in processes that lead to pulmonary fibrosis formation and pulmonary remodeling, as well as in eosinophil recruitment and mucus production.⁶ Periostin has been evaluated in the context of pulmonary fibrosis and asthma, but a recently published study revealed no significant difference between the levels of periostin in asthma compared to in COPD.³⁰ Several studies suggested that periostin

is a biomarker in patients with IPF,^{6,7} but no studies have evaluated periostin behaviour in CPFE. Caswell-Smith et al. reported reference values for periostin in adults without asthma and no COPD at 50 ng/mL.³⁰ In our study, only the CPFE group showed values higher than the reference. As this biomarker may be increased in both fibrosis and COPD, it is possible that the association of the two changes contributed to the outstanding increase in patients with CPFE.

Mucin-16 (MUC16 or CA-125) is a tumor biomarker widely evaluated in medical practice and is mainly related to gynecological tumors, such as those in the ovaries.^{12,13} However, some authors observed increased levels (>35 U/mL) in patients with interstitial lung diseases, particularly IPF, even suggesting a worse prognosis with increased mortality and increased risk of lung cancer.^{12,13} We found that patients with IPF had higher levels of mucin-16 than patients with CPFE. This may be related to the fibrosis degree. One hypothesis to explain this results is that the presence of emphysema reduces lung density, as mucin-16 is present on the pulmonary epithelial surface.

PDGF-BB and TGF- β_1 have been proposed as biomarkers for differentiating between IPF and CPFE. These molecules participate in collagen formation and complement system regulation⁹⁻¹¹ and may help in this differentiation; however, the data revealed no significant difference in PDGF-BB and TGF- β_1 among the three groups.

Because of the cross-sectional design of the study, the prognostic role of these functional and biochemical markers could not be assessed. Another limitation of this study was that the study was conducted in a single center and included only patients with an evident UIP pattern, and it was not possible to evaluate whether the observed changes also occurred in patients with incipient lesions. In addition, the lack of a control group with healthy patients may be a limiting factor. However, this study provides insight useful for future studies that include patients with IPF and CPFE.

Conclusions

SBWN₂ is an easy test to perform and may assist in the differential diagnosis of IPF and CPFE. Additionally, periostin appears to be an important biomarker for differentiating CPFE from IPF, just as mucin-16 appears to be related to IPF. These data suggest that CPFE has distinct functional and serologic characteristics from isolated IPF.

List Of Abbreviations

CC: Closing capacity

CO: Carbon monoxide

COPD: Chronic obstructive pulmonary disease

CPFE: Combined pulmonary fibrosis and emphysema

CV: Closing volume

DLco: Carbon monoxide diffusion capacity

ELISA: Enzyme-linked immunosorbent assay

FEV₁: Forced expiratory volume in the first second

FVC: Forced vital capacity

GOLD: Global Initiative for Chronic Obstructive Lung Disease

HRCT: High resolution chest computed tomography

ILD: Interstitial lung disease

IPF: Idiopathic pulmonary fibrosis

IQR: interquartile range

Kco: Transfer coefficient

MDD: Multidisciplinary discussion

mMRC: Modified Medical Research Council

MUC-16: Mucin-16

PDGF-BB: Platelet-derived growth factor-BB

RV: Residual volume

SIII: Phase III slope

SBWN₂: Single-maneuver nitrogen washout test

TGF-β₁: Transforming growth factor

TLC: Total lung capacity

UIP: Usual interstitial pneumonia

VC: vital capacity

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Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Pedro Ernesto University Hospital, State University of Rio de Janeiro.

Consent for publication

All research subjects signed and obtained a copy of the Free and Informed Consent Form.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests in this section.

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Authors' contributions

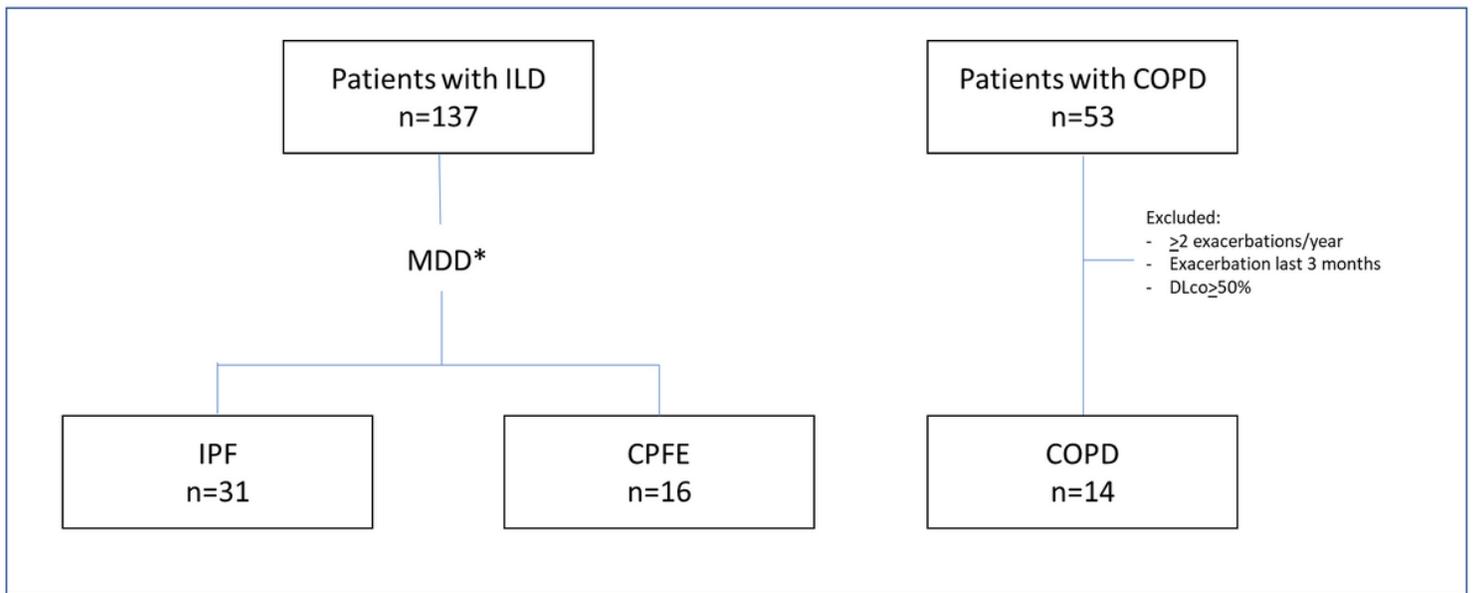
FMA did the historical reference, collected the data, analyzed and interpreted the data and wrote the article. CHC assisted in the historical framework, in the analysis and interpretation of data and in the writing of the article. LSR, TPA, MMA and MCBSL performed the analysis of laboratory tests. LFF and TFC assisted in data collection. AGL and RLR assisted in the analysis and interpretation of the data.

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Not applicable.

Figures

Figure 1: Flow diagram design



(*) After MDD, cases recommended to biopsy or suggestive of an alternative diagnosis were excluded. Only cases with tomographic UIP pattern and a firm diagnosis of IPF and CPFE were included.

ILD: interstitial lung disease, MDD: multidisciplinary discussion, IPF: idiopathic pulmonary fibrosis; CPFE: combined pulmonary fibrosis and emphysema; COPD: chronic obstructive pulmonary disease, DLco: Carbon monoxide diffusion capacity

Figure 1

Flow diagram design

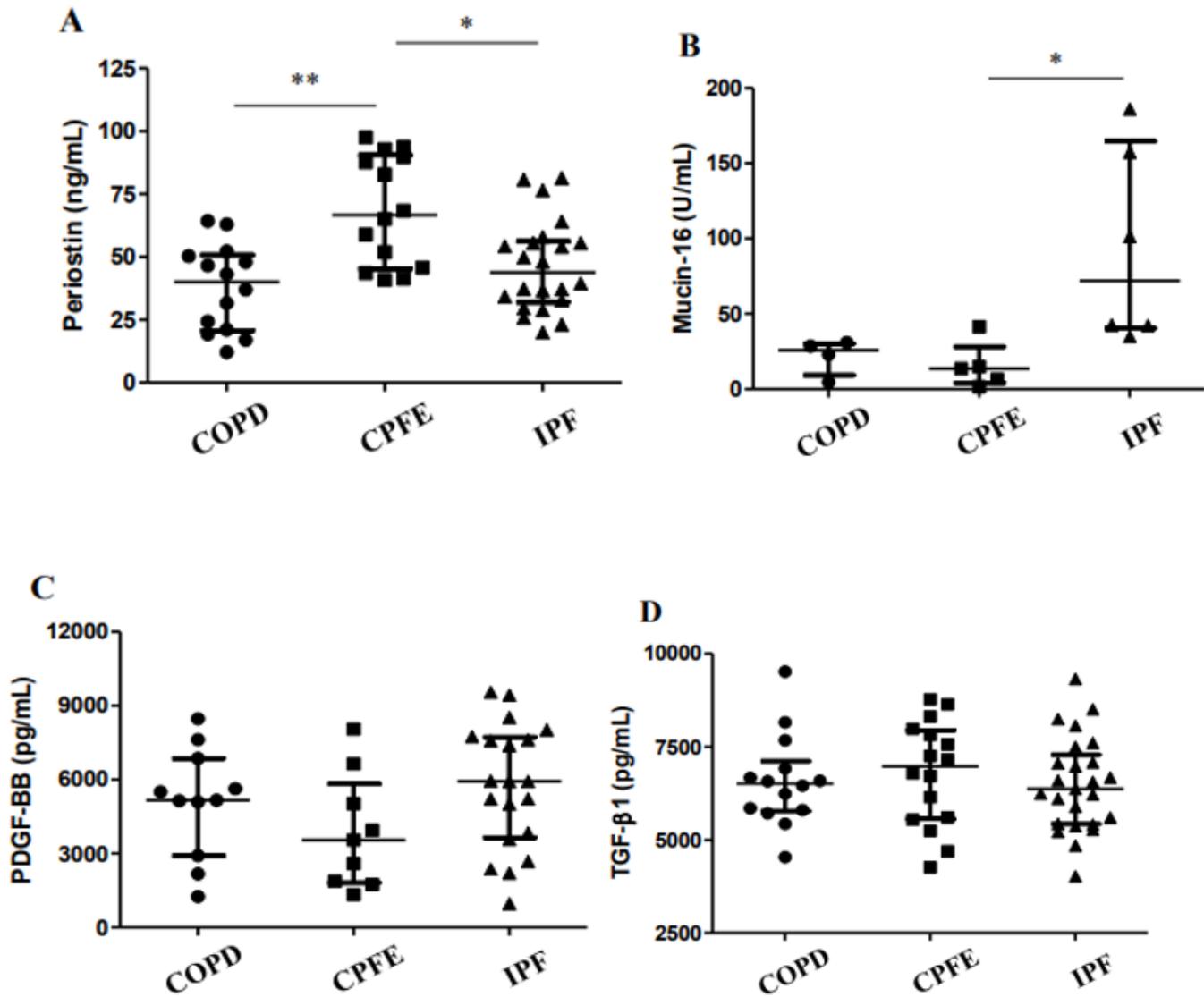


Figure 2

Serum biomarkers evaluated in patients with IPF, CPFE and COPD. Circulating levels of periostin (A), mucin-16 (B), PDGF-BB (C) and TGF-β1 (D) from patients diagnosed with pulmonary emphysema, CPFE and IPF. The biomarkers were measured in sera of patients using ELISA. * $p < 0.05$; ** $p < 0.001$. CPFE: combined pulmonary fibrosis and emphysema; IPF: idiopathic pulmonary fibrosis.