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ORIGINAL RESEARCH

Diagnostic Values For Club Cell Secretory Protein (CC16) in Serum of Patients of Combined Pulmonary Fibrosis and Emphysema

Nariaki Kokuho,[1,2] Takeo Ishii,[1,2] Koichiro Kamio,[1,2] Hiroki Hayashi,[1,2] Misuzu Kurahara,[1,2] Kumiko Hattori,[1,2] Takashi Motegi,[1,2] Arata Azuma,[2] Akihiko Gemma,[2] and Kozui Kida[1,2]

1 Respiratory Care Clinic, Nippon Medical School, Tokyo, Japan

2 The Department of Pulmonary Medicine and Oncology, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan

Abstract

Combined pulmonary fibrosis and emphysema (CPFE) is an under-recognized syndrome for which the diagnostic use of serum biomarkers is an attractive possibility. We hypothesized that CC16 and/or TGF-β1 or combinations with other biomarkers are useful for diagnosing CPFE. Patients with respiratory symptoms and a smoking history, with or without chronic obstructive pulmonary disease, were divided into the following three groups according to findings of high-resolution computed tomography of the chest: controls without either emphysema or fibrosis, patients with emphysema alone, and patients compatible with the diagnosis of CPFE. Serum concentrations of CC16, TGF-β1, SP-D, and KL-6 were measured in patients whose condition was stable for at least 3 months. To investigate changes in biomarkers of lung fibrosis in patients with a life-long smoking history, additional measurements were performed on the patients with idiopathic pulmonary fibrosis (IPF) of smoking history. The mean age of the first three groups was 68.0 years, whereas that of the IPF group was 71.8 years, and the groups contained 36, 115, 27, and 10 individuals, respectively. The serum concentration of CC16 in the four groups was 5.67 ± 0.42, 5.66 ± 0.35, 9.38 ± 1.04 and 22.15 ± 4.64 ng/ml, respectively, indicating that those patients with lung fibrosis had a significantly higher concentration. The combined use of CC16, SP-D, and KL-6 provided supportive diagnosis in conjunction with radiological imaging in diagnosis of CPFE. We conclude that a combination of biomarkers including CC16 could provide useful information to screen and predict the possible diagnosis of CPFE.

Keywords: club cell secretory protein, combined pulmonary fibrosis and emphysema (CPFE), diagnosis, emphysema, lung fibrosis

Correspondence to: Kozui Kida, M.D., Respiratory Care Clinic, Nippon Medical School, 4-7-15-8F, Kudan-minami, Chiyoda-ku, Tokyo 102-0074, Japan, phone: +81-3-5276-2325, fax: +81-3-5276-2326, email: kkida@nms.ac.jp

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Abbreviations

CPFE: combined pulmonary fibrosis and emphysema; CC16: club cell secretory protein; TGF-β1: transforming growth factor beta 1; COPD: chronic obstructive pulmonary disease; IPF: idiopathic pulmonary fibrosis; AUC: areas under the curve; KL-6: Krebs von den Lungen 6; SP-D: surfactant protein D; 6MWT: 6-minute walking tests; HRCT: high-resolution computed tomography; LAA: low attenuation area; ANOVA: analyses of variance; ROC: Receiver Operator Characteristic; GOLD: global initiative for chronic obstructive lung disease; FVC: forced vital capacity; FEV1: forced expiratory volume in one second; TLC: total lung capacity; RV: residual volume; DLCO: diffusing capacity of the lung; VA: alveolar volume; $PaO_{2:}$ partial pressure of oxygen in arterial blood; $PaCO_2$:

partial pressure of carbon dioxide in arterial blood; $AaDO_2$: alveolar-arterial oxygen difference; LVEF: left ventricular ejection fraction; PAP: pulmonary artery pressure.

Introduction

Historically, the presence of excess fibrosis has been thought to preclude the diagnosis of emphysema (1). In histopathological analysis, obvious fibrosis has been excluded from the diagnostic definition of emphysema, but increased collagen content in emphysemic lungs has been reported by some studies (2–6). These data suggest that emphysema may be accompanied with fibrosis to some extent, despite appearing without obvious fibrosis under a light microscope.

In 2005, Cottin and associates reported a new clinical entity: combined pulmonary fibrosis and emphysema (CPFE), which is typically characterized by upper lobe emphysema and pulmonary fibrosis of the lower lungs (7). Subsequently, several groups made a series of reports that support CPFE as a distinct syndrome (8–10). Although a similar concept was reported earlier (11), the notion of CPFE opened up a new perspective on chronic obstructive pulmonary disease (COPD) and interstitial lung disease, particularly idiopathic pulmonary fibrosis (IPF), which is being actively studied in terms of new therapeutic regimens (12) or new genetic associations (13). CPFE syndrome has characteristic imaging features and consists of a combination of distinct signs and symptoms, which include severe dyspnea; physiological testing reveals normal lung volume indices with markedly impaired diffusion capacity, hypoxemia during exercise (14) and, occasionally, pulmonary hypertension (15, 16).

CPFE, however, has not yet been recognized widely, and more studies are needed to determine the entire clinical and basic features of CPFE (17). Currently, serum biomarkers for lung diseases are an active area of research, but finding a biomarker useful for diagnosis or prognosis is a major challenge. Club cell secretory protein (CC16) and/or transforming growth factor β1 (TGF-β1) show promise as serum markers for assessing the cellular integrity or permeability of the lung structure (18, 19). Accordingly, we aimed to find specific biomarkers that ideally would differentiate CPFE from uncomplicated emphysema in daily practice. We tested the hypothesis that CC16, TGF-β1, and combinations with other promising biomarkers are useful for differential diagnosis of CPFE.

Methods

Study design and patients

We enrolled 410 consecutive patients with long-term smoking history who had visited the Respiratory Care Clinic, Nippon Medical School, Tokyo, Japan, from November 2003 to March 2008 regarding expectoration or chronic cough and/or dyspnea during exercise. Detailed information on eligibility and exclusion criteria is available in the Supplementary Material found online.

To investigate changes in CC16 in lung fibrosis with life-long smoking history, 10 smokers with idiopathic pulmonary fibrosis (IPF) who visited the university hospital, Nippon Medical School, Tokyo, Japan, were recruited. The diagnosis of IPF was confirmed after detailed examinations in accordance with the criteria of ATS/ERS/JRS/ALAT statement (20).

This study was conducted in accordance with the Declaration of Helsinki, and all patients provided written informed consent. The study protocol was approved by the Institutional Review Board of Nippon Medical School, Tokyo, Japan.

Study procedures (Detailed information is available in the Supplementary Material)

At first visit, the patients underwent examinations as follows: post-bronchodilator pulmonary function tests, diffusion capacity, arterial blood gas with room air breathing, and 6-minute walking tests (6MWT). High-resolution computed tomography (HRCT) scanning of the chest was performed, followed by a quantitative assessment of the extent of emphysema (%LAA) using software. Echocardiography was performed by a technician, under the supervision of a qualified cardiologist to assess pulmonary hypertension or left ventricular heart failure.

Subgroups and biomarkers

Severity classification of COPD was based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (21). We defined CPFE according to the original report by Cottin and colleagues (7) as a syndrome characterized by upper lobe emphysema and pulmonary fibrosis of the lower lungs according to HRCT of the chest. The latter images were reviewed separately by a radiologist and two of the authors who did not have access to any information on the clinical status of the study patients. A fourth opinion was sought if there was no clear consensus.

According to the chest HRCT findings, all individuals were divided into three groups: control smokers who had neither emphysema nor fibrosis; patients who had emphysema alone; and patients with CPFE who had upper-lobe–dominant emphysema and lower-lobe–dominant fibrosis. A fibrotic score was determined according to the method described by Kazerooni and colleagues (22).

Serum samples for biomarker measurements were stored at −80°C until biomarker assays. Analysis of serum concentrations of transforming growth factor β1 (TGF-β1), surfactant protein D (SP-D), club cell secretory protein 16 (CC16), and Krebs von den Lungen 6 (KL-6) was conducted in the three groups and 10 patients with IPF as described in the Online

Supplement. The data for each biomarker were compared among the three groups, and the same procedure was followed for combinations of 2–3 biomarkers. In order to exclude the effects of renal insufficiency, creatinine concentration was measured in all serum samples; we excluded samples in which the creatinine concentration exceeded the reference range.

Statistical analysis

Comparison among the three groups was carried out by means of analyses of variance (ANOVA) or Kruskal–Walls tests as appropriate; *t*-tests based on the appropriate linear combinations of the random effects, and their standard errors were used to compare the means. When *p* values were less than 0.05, the differences were considered statistically significant. Adjustments were made for age, sex, smoking status, and blood creatinine levels. The ability to classify a group was assessed using the C statistic (23). The overall C statistic is defined as the probability of concordance among groups that can be compared. The biomarkers can be compared if it can be determined which one is suitable for detecting CPFE.

The C statistic was calculated as the sum of concordance values divided by the number of comparable pairs among all or several biomarkers. In addition, Receiver Operator Characteristic (ROC) curves were plotted for the diagnostic models of the biomarkers. All analyses were conducted using the JMP software, version 9.0.3 for Windows (SAS Institute Inc.). Additional details about the statistics can be found in the Online Supplement.

Results

A flow diagram of the study design and the selection process for eligible patients is shown in Figure 1. Ultimately, 178 individuals were divided into the following three groups: smoker control, emphysema alone, and CPFE, consisting of 36, 115, and 27 individuals, respectively.

The baseline characteristics of the patients, which include the data on severity of COPD according to the GOLD criteria, are shown in Table 1 and Table 2 (only key parameters are shown here, and detailed data that include various other measurements are available in the *Online Supplement* in ETables 1–3). The CPFE group was significantly older ($p < 0.0001$), showing greater prevalence of a smoking history ($p = 0.0005$), a smaller distance in 6MWT with significant arterial desaturation ($p < 0.0001$) compared with the smoker control or emphysema alone groups.

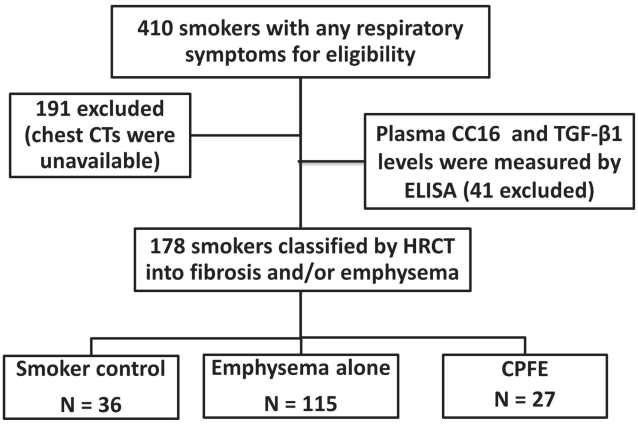

Figure 1. A flow diagram of study design. CC16: club cell protein 16; TGF-β1: transforming growth factor β1; ELISA: enzyme-linked immunosorbent assay; CPFE: combined pulmonary fibrosis and emphysema.

With regard to the pulmonary function, the total lung capacity was the lowest in the CPFE group ($p = 0.0015$), whereas the emphysema alone group showed greater airflow obstruction. The mean %LAA in the upper lung field was similar in the emphysema alone group, whereas in the CPFE group %LAA was significantly larger than that of the smoker control group ($p < 0.0001$). The mean fibrotic score in the CPFE group was 8.9 ± 5.7 (Kazerooni's score range: no lung fibrosis to maximum, 0–30). Serum creatinine levels were within normal limits and closely correlated with the serum concentration of CC16 for all individuals ($p < 0.00001$); however, no association was found between the CC16 concentration and carbon monoxide in blood air.

The serum concentration of CC16 in the smoker control, emphysema alone, and CPFE groups was 5.67 ± 0.42,

Table 1. Basic characteristics of the three groups

	Smoker control (n = 36)	Emphysema alone (n = 115)	CPFE (n = 27)	All (n = 178)	*p* value
Age, years	64.5 ± 10.3	67.4 ± 8.9	74.9 ± 7.7	68.0 ± 9.5	<0.0001
Gender, M/F	30/6	109/6	24/3	163/15	NS
Pack-years	42.5 ± 27.8	78.7 ± 47.2	91.1 ± 56.8	73.5 ± 47.9	0.0005
COPD/non-COPD	15/21	104/11	14/13	133/45	<0.0001
GOLD I/II/III/IV	6/6/3/0	10/42/41/11	6/4/4/0	22/52/48/11	0.0042
Average upper LAA	16.6 ± 11.8	38.5 ± 15.7	30.9 ± 18.1	32.9 ± 17.6	<0.0001

The data were analyzed using ANOVA and are presented in the table as mean ± SD (range). M: male, F: female, COPD: chronic obstructive pulmonary disease, GOLD: global initiative for chronic obstructive lung disease, LAA: low attenuation area and NS: not significant.

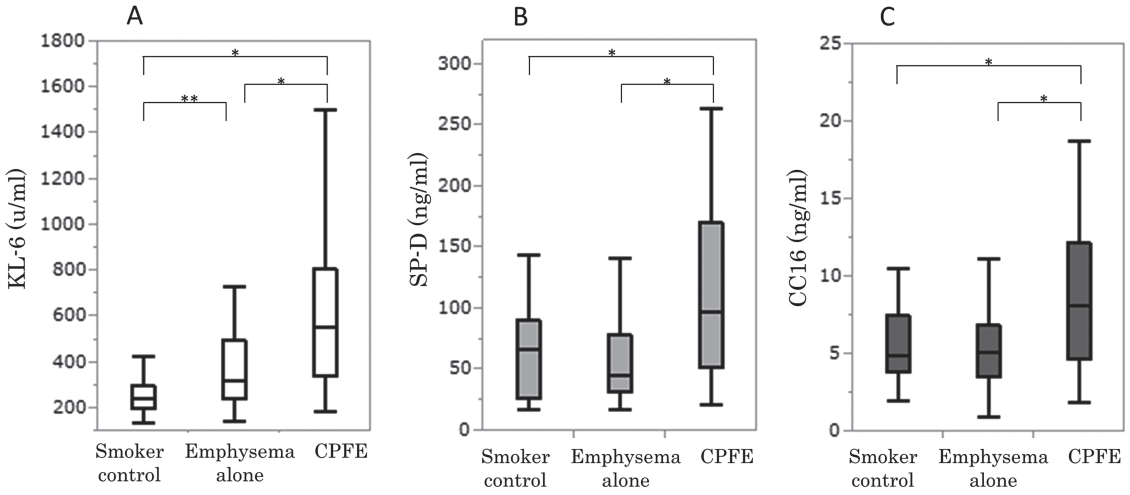

Figure 2. Box-plot of the biomarkers among smoker control, emphysema alone and CPFE groups. (2A): serum concentrations of KL-6 among three groups ($p < 0.0001$). (2B): serum concentration of SP-D among three groups ($p = 0.001$). (2C): serum concentration of CC16 among three groups ($p = 0.0009$). KL-6: Krebs von den Lungen-6; SP-D: surfactant protein D; CC16: club cell secretory protein 16; CPFE: combined pulmonary fibrosis and emphysema. $^{*}p < 0.01$, $^{**}p < 0.05$, when compared between groups.

5.66 ± 0.35, and 9.38 ± 1.04 ng/mL, respectively, and it was highest in the CPFE group ($p = 0.0009$, Figure 2). No significant difference was observed in serum concentrations of TGF-β1 among the three groups; the concentrations were 37.5 ± 1.9, 42.6 ± 2.4, and 37.2 ± 2.6 ng/ml in the smoker control, emphysema alone, and CPFE groups, respectively. Furthermore, significant differences were observed among the groups in serum concentrations of KL-6, SP-D, and CC16; a significant association was found between CC16 and SP-D after logarithmic transformation ($p = 0.01$) for all individuals.

In addition, we investigated the association between these biomarkers and various clinical parameters in the CPFE group. The percent predicted forced expiratory volume in 1 second (FEV_1) was significant and exhibited a positive correlation with SP-D ($p = 0.016$; $R^2 = 0.217$); similarly, mean systolic pulmonary pressure (sPAP) showed a positive correlation with SP-D ($p = 0.041$; $R^2 = 0.202$). However, there was neither a significant correlation between all three biomarkers nor with the percent forced vital capacity (FVC) or fibrotic scores.

The C statistics for the model of CPFE diagnosis for the three biomarkers, KL-6, SP-D, and CC16, were 0.733, 0.724, and 0.743, respectively. Areas under the curve (AUC) of the ROC curves to detect CPFE were 0.828 (95% CI, 0.721-0.899), 0.804 (95% CI, 0.691-0.883) and 0.813 (95% CI, 0.698-0.891) for KL-6 and CC16, SP-D and CC16, and the combination of KL-6, SP-D, and CC16, respectively (Figure 3). However, there were no significant differences in AUC among all three combinations. The data on sensitivity, specificity, AUC for the combinations (KL-6 and CC16, SP-D and CC16, and all three combined), and the threshold for each biomarker (KL-6, SP-D, and CC16) are presented in ETable 4 in the *Online Supplement*.

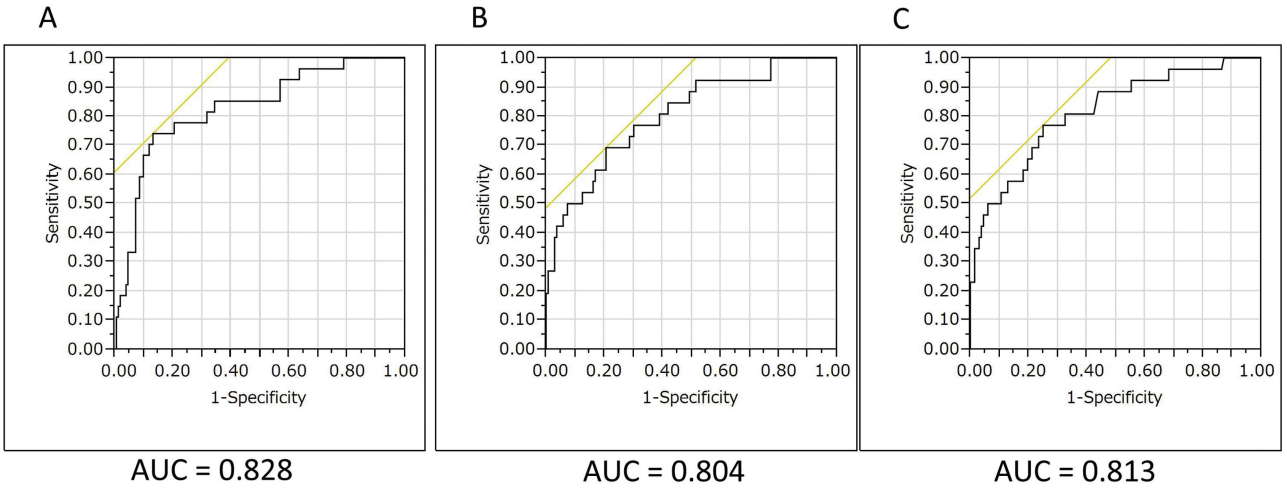

Figure 3. Receiver Operator Characteristic (ROC) analysis of the three combinations of biomarkers: The AUC for each combination was as follows: KL-6 and CC16 (Fig 3A), SP-D and CC16 (Fig 3B), KL-6, SP-D, and CC16 (Fig 3C) was 0.828, 0.804 and 0.813, respectively. Details of ROC analysis are provided in ETable 5. The combination of KL-6 and CC16 appeared better than the other two combinations. KL-6: Krebs von den Lungen 6, CC16: club cell secretory protein 16, SP-D: surfactant protein D, AUC: area under the curve.

Table 2. Clinical manifestations

	Smoker control (n = 36)	Emphysema alone (n = 115)	CPFE (n = 27)	All (n = 175)	*p* value
FVC %	3.58 ± 0.93	3.29 ± 0.82	3.07 ± 0.75	3.32 ± 0.84	NS
FEV_1/FVC %	73.7 ± 9.3	51.7 ± 13.7	68.7 ± 18.4	58.7 ± 16.8	<0.0001
FEV_1 % predicted	93.6 ± 20.2	60.3 ± 22.3	82.4 ± 24.1	70.4 ± 26.1	<0.0001
DLCO %	112.6 ± 23.5	75.7 ± 22.8	62.4 ± 21.9	81.2 ± 28.1	<0.0001
PaO_2 at rest	89.2 ± 8.5	81.5 ± 9.5	85.1 ± 10.3	83.7 ± 9.9	0.0002
$PaCO_2$ at rest	40.9 ± 3.3	39.8 ± 4.1	41.4 ± 3.0	40.2 ± 3.8	NS
$AaDO_2$	9.7 ± 8.4	18.8 ± 10.9	13.2 ± 10.7	16.0 ± 11.0	<0.0001
Distance, 6MWT	527.4 ± 68.8	469.3 ± 86.2	426.1 ± 87.8	474.5 ± 88.3	<0.0001
ΔSpO_2	0.9 ± 1.2	4.8 ± 5.0	5.5 ± 5.3	4.1 ± 4.8	<0.0001
Borg Scale	1.8 ± 1.5	3.1 ± 2.0	3.1 ± 2.1	2.9 ± 2.0	0.0019
LVEF	69.7 ± 5.0	68.5 ± 6.1	68.5 ± 5.7	68.8 ± 5.8	NS
Systolic PAP#	30.7 ± 5.2	34.9 ± 6.9	35.0 ± 8.3	34.1 ± 7.0	0.0127

The data were analyzed using ANOVA and are presented in the table as mean ± SD (range). FVC: forced vital capacity, FEV_1: forced expiratory volume in one second, DLCO: diffusing capacity of the lung, PaO_2: partial pressure of oxygen in arterial blood, $PaCO_2$: partial pressure of carbon dioxide in arterial blood, $AaDO_2$: Alveolar-arterial oxygen difference, 6MWT: 6-minute walking test, LVEF: left ventricular ejection fraction, PAP: pulmonary artery pressure, #: assessed by echocardiogram and NS: not significant.

The mean age of the IPF group was 71.8 years. The results of the pulmonary function tests in this group showed a mild restrictive disorder and severe diffusion disturbance. The mean fibrotic score in these patients with IPF was higher than that of the CPFE patients (12.7 ± 5.0 vs. 8.9 ± 5.7). The serum concentrations of CC16, KL-6, and SP-D were 22.15 ± 4.64 ng/ml, 1128.8 ± 556.3 U/ml, and 134.9 ± 68 ng/ml, respectively, in the patients with IPF, and the CC16 concentration was higher in the IPF group than in the CPFE group.

Detailed data that include various other measurements are available in ETable 5 in the *Online Supplement*.

Discussion

In the present study, we have shown that the serum concentration of CC16 increases in patients with CPFE and that combined testing for KL-6 and CC16 can effectively differentiate CPFE from emphysema alone. The diagnosis of usual or classical interstitial pneumonia (UIP) is made using a required histopathological assessment since the seminal report by Liebow and Smith in 1968 (24). More recently, imaging analysis such as high-resolution computed tomography (HRCT) has shown remarkable development, and UIP can now be detected in HRCT images (12). Nonetheless, some cases are difficult to diagnose even using both histopathological evaluation and an advanced imaging technique; therefore, a different (simpler) diagnostic method is needed.

Increased concentration of CC16

The 15.8 kDa club cell protein (CC16) is the major protein secreted by club cells and one of the main secretory proteins in the lung. CC16 occurs at high concentrations in the epithelial lining fluid where it appears to play an antioxidant/anti-inflammatory role mostly by modulating the production and/or activity of phospholipase A2, interferon-γ, and tumor necrosis factor α (18, 25, 26). A previous report has shown that serum CC16 levels are significantly elevated in idiopathic interstitial pneumonia (27), which is consistent with the data in the present study.

Similarly, in cases of scleroderma or sarcoidosis, which is associated with interstitial pneumonia, the extent of fibrosis significantly correlates with the level of CC16 (28-30). The results of the present study suggest that the increased level of CC16 in CPFE might reflect the degree of lung inflammation and/or fibrosis. It is unknown whether the elevated CC16 concentration is a transient phenomenon in CPFE or whether it is related to transient inflammation in the lung tissue.

Lakind and colleagues (31) examined the relationship between serum CC16 and asthma etiology and exacerbations, and they found that acute exposure to certain pulmonary irritants or localized pulmonary inflammation can cause a transient increase in the serum CC16 level. They also showed that a transient increase in serum CC16 is not associated with detectable pulmonary damage or impairment of pulmonary function (31). In this regard, changes in the serum CC16 concentration during follow-up for CPFE, if they exist, might be not only a diagnostic predictor but also a therapeutic biomarker; further research concerning this use of CC16 is needed.

Effects of smoking on CC16

The data on CC16 in the smoker control and emphysema alone groups are also interesting. Chronic smoking reduces the serum concentration of CC16 in a dose-dependent manner; this effect is associated with

a concomitant reduction of CC16 in lung lavage and a progressive decline of club cell numbers (18). This observation also suggests that cessation of smoking restores the serum concentration of CC16 (18). In one follow-up study of COPD patients, the serum concentration of CC16 at baseline correlated with a slower rate of decline of FEV_1 (32).

Lomas and coworkers showed that the median serum CC16 level is significantly lower for current and former smokers with COPD than for current and former smokers with no airflow obstruction in a *post hoc* analysis of the ECLIPSE study (33). The smoking status should affect the concentration of CC16, but this concentration appears to be close to normal in the smoker control group, including current and ex-smokers. There are several possible explanations. Data on the smoking duration for each patient in the present study was obtained from self-reports (which can be inaccurate), and smoking status and/or duration might affect the serum CC16 concentration after smoking cessation; thus, prospective research is useful and necessary in this regard.

We hypothesize that club cell activity decreases in emphysemic lungs because of the loss of bronchioles in the pathogenesis of COPD (34), whereas the activity of club cells might increase when emphysema coexists with fibrosis in a different part of the same lung (as in CPFE), in keeping with the findings outlined above. Although it is intriguing that the activity of club cells in emphysema and lung fibrosis is different and may change in opposite directions, further investigation is needed to clarify the physiological basis for this phenomenon.

Other serum biomarkers in lung fibrosis

Krebs von den Lungen 6 (KL-6) shows promise as a diagnostic marker of interstitial lung diseases (26). Serum KL-6 is elevated in the majority of patients with interstitial lung diseases relative to patients with bacterial pneumonia and healthy individuals (35). KL-6 levels depend on the number of regenerating type II epithelial cells and the integrity of the alveolar–capillary membrane (36). Because KL-6 is chemotactic for human fibroblasts, this protein may also play a functional role in fibrosis (37).

The levels of KL-6 were significantly different ($p = 0.02$) between smoker control and emphysema alone groups. This observation was consistent with a previous report showing that the serum concentration of KL-6 was higher in patients with emphysema versus healthy control individuals (38). The present data show that the combination of biomarkers CC16 and KL-6 was the best predictor for CPFE (AUC = 0.828); however, the combination of three promising biomarkers (CC16, KL-6, and SP-D) was the second best (AUC = 0.813). The two values are close, suggesting that the triple combination should not be discarded and can still be considered useful for the diagnosis of CPFE; this possibility needs further research in a large cohort study.

In order to investigate changes in CC16 levels in patients with the combined effects of lung fibrosis and life-long smoking, we studied an additional ten patients with IPF. The data show that the serum concentration of CC16 in the IPF group was significantly higher that of the CPFE group. We had hoped to be able to compare the data of patients with IPF and CPFE as a case control study, ideally in patients with similar fibrotic scores; however, we were unable to recruit such patients for the present study.

Despite this deficit, the present data provide useful information for further understanding role of CC16 in the CPFE and IPF groups. A higher serum concentration of CC16 in the IPF group might simply be a reflection of fibrotic changes *per se*, similarly that in the CPFE group. If this speculation were correct, the pathogenesis of lung fibrosis in CPFE and IPF would be similar as has been previously reported (39). Further studies are needed to confirm this hypothesis.

CPFE is defined as a clinical diagnosis based mainly on characteristic radiological findings (7). However, the CC16 data in this study might provide additional diagnostically relevant information, since serum concentrations of CC16 are decreased in patients with chronic lung damage caused by tobacco smoke and other air pollutants as a consequence of the destruction of club cells.

Characteristics of the study design and limitations

Among the enrolled cases in the present study, we carefully selected cases compatible with the criteria described in the original report by Cottin et al. (7). In CPFE patients in the present study, the mean age was 74.9 versus 65.2 years in the previous study (7); never-smokers were not examined in both studies. Also not examined in either study were HRCT imaging for pulmonary interstitial shadows, which are consistent with idiopathic pulmonary fibrosis (IPF), and data on pulmonary function during mild restrictive and obstructive ventilator disturbance.

A decrease in walking distance during a 6-minute walking test (and slight arterial desaturation during the test) as well as an increase in mean pulmonary arterial pressure in echocardiograms were observed in both studies; our data are consistent with those by Cottin et al. (7) and others (9, 10, 14–17). By definition, CPFE is emphysema in the upper lobe and interstitial pneumonia in the lower lobe (7), but there are many cases with comorbidities and a mixed clinical picture, and the degree of pathological changes that are diagnostic of CPFE have yet to be clearly defined. Moreover, interstitial pneumonia is diverse; the degree of disease activity at the tissue level in the same lung is not uniform. Under such circumstances, in the CPFE cases where fibrosis and emphysema are mixed to various degrees, for clinical diagnosis, physicians can assume that combinations including CC16 are reliable.

The fibrotic score of CPFE in the present study was suggestive of mild fibrosis, which might result in a mild increase in serum concentrations of both KL-6 and SP-D, whereas the emphysema score, which was assessed by %LAA, indicated moderate emphysema.

It would be interesting to conduct a study that compares diagnostic power for CPFE between chest HRCT and biomarkers of other types of interstitial fibrosis. We did not evaluate the influence of medication in this study, such as inhaled corticosteroids (this influence needs to be assessed in future research).

Although the present study addressed only the diagnostic role of serum biomarkers among the patients with CPFE alone, which were compatible with the criteria of the original report (7), these new data on CC16 in CPFE might shed some light on the pathogenesis of this disease.

Conclusions

Combinations of biomarkers including CC16 can provide useful information for screening and predicting CPFE, in addition to lung imaging data.

Acknowledgments

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Declaration of Interest Statement

All authors have declared that they have no conflicts of interest regarding this work. The authors are responsible for the writing and the content of this paper.

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