Increased Levels of Osteopontin in Sputum Supernatant in Patients With COPD

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BACKGROUND: Osteopontin (OPN) is a phosphorylated acidic glycoprotein that can function as both an extracellular matrix molecule and a cytokine. Published data support that OPN is upregulated in surgical lung tissue samples of patients with COPD. The aim of this study was to determine the levels of OPN in sputum supernatants of patients with COPD and to investigate possible associations with mediators and cells involved in the inflammatory and remodeling process as well as with the extent of emphysema.

METHODS: Seventy-seven patients with COPD and 40 healthy subjects (20 smokers) were studied. All subjects underwent lung function tests, sputum induction for cell count identification, and OPN, transforming growth factor-β_1, matrix metalloproteinase (MMP)-2, IL-8, and leukotriene-4 measurement in sputum supernatants. High-resolution CT (HRCT) scan of the chest was performed for quantification of emphysema.

RESULTS: OPN levels (pg/mL) were significantly higher in patients with COPD compared with healthy smokers and nonsmokers (median [interquartile range], 1,340 [601, 6,227] vs 101 [77, 110] vs 68 [50, 89], respectively; $P < .001$). Regression analysis showed a significant association between OPN and sputum neutrophils, IL-8, MMP-2, and the extent of emphysema. The associations previously listed were not observed in healthy subjects.

CONCLUSIONS: OPN levels are higher in patients with COPD compared with healthy subjects. OPN may play a role in the neutrophilic inflammation and in the pathogenesis of emphysema.

ABBREVIATIONS: D_{LCO} = diff using capacity of the lung for carbon monoxide; ELISA = enzyme-linked immunosorbent assay; HRCT = high-resolution CT; LTB4 = leukotriene B4; MMP = matrix metallo-proteinase; OPN = osteopontin; TGF = transforming growth factor

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COPD is a destructive lung disorder characterized by inflammatory and tissue remodeling processes that is associated with a chronic enhanced inflammatory response in the airways and the lung to noxious particles or gases. Pathologic features of COPD are pulmonary inflammation, remodeling-like changes in mucosal lung tissue, fibrosis, and tissue injury.[1] Although considerable information has been gathered concerning the role of different agents in the promotion of inflammation and airway destruction in the acute setting of the disease, relatively little is known about signaling pathways that drive their progressive and chronic nature.[2]

Osteopontin (OPN) is a phosphorylated acidic glycoprotein that can function both as an extracellular matrix molecule and as a cytokine. Its origin is not well clarified, but epithelial cells, macrophages, T cells, and fibroblasts have been shown to express OPN. Limited data are available regarding OPN in COPD. Adenosine deaminase-deficient mice develop alveolar airspace enlargement that resembles histologic findings of patients with emphysema.[3] OPN has been found to be associated with alveolar airspace enlargement, neutrophilia, and elevations of mediators of airspace enlargement such as matrix metalloproteinases (MMPs) in adenosine deaminase-deficient mice.[3,4] Given that the majority of OPN immunolocalization in human COPD lung samples was in alveolar macrophages and that OPN expression was associated with the severity of airflow obstruction, it was suggested that OPN may be a major mediator of emphysema.[4] We have reported that sputum OPN levels are higher in patients with asthma who smoke compared with patients with asthma who are nonsmokers and in patients with severe refractory asthma compared with less severe forms of the disease.[5,6]

The aim of the present study was to evaluate the levels of OPN in sputum supernatants of patients with COPD in comparison with healthy smokers and nonsmoking subjects. We hypothesized that sputum OPN would be elevated in patients with COPD compared with healthy control subjects and that it would be associated with inflammatory cells and mediators involved in the ongoing airway inflammation process observed in COPD. We also hypothesized that OPN levels would be associated with the extent of emphysema.

Materials and Methods

Subjects

Patients with COPD were recruited aft er extensive evaluation from an open cohort of patients with COPD who were followed in the outpatient clinics of the 1st and 2nd Respiratory Medicine Departments of the University of Athens Medical School. Initially, 94 patients were evaluated. The diagnosis of COPD and the severity of airflow obstruction was established according to the GOLD (Global Initiative for Chronic Obstructive Lung Disease) guidelines.[7] Patients had a history of smoking (> 20 pack-years) and irreversible airflow limitation (reversibility < 12% and < 200 mL in FEV_1 aft er β_2-agonist inhalation). Subjects with a malignant disease were excluded. All patients were clinically stable, with no evidence of acute exacerbation for at least 4 weeks prior to enrollment. Twenty healthy nonsmokers and 20 healthy smokers served as control subjects. Th e healthy subjects were recruited from the staff of both departments and have also been used as a control group in previous studies of our group.[5] Th e study was approved by the ethics committees of both hospitals (Sotiria Hospital and Attikon Hospital Ethics Committee No:517/May 2008), and all subjects provided written informed consent.

Study Design

On day 1, all subjects underwent medical history and physical examination by a dedicated respiratory physician, lung function measurements, and measurement of BMI, and all patients with COPD underwent high-resolution CT (HRCT) scan of the chest. On day 2, sputum induction was performed. All measurements were performed between 8.00 AM and 10.00 AM. All smokers refrained from smoking for at least 2 h, under supervision of one of the study coordinators. HRCT scans were performed as part of the medical assessment of patients with COPD. Patients with fi ndings suspicious for malignancies or nondefi ned conditions were excluded.

Induced Sputum

Sputum was induced as previously described, using all necessary modifications for safe measurements according to the underlying airflow obstruction.[8] Sputum was processed using selected plugs as previously described.[9] Dithiothreitol was added in a volume equal to four times the weight of the sputum specimen, and it was further diluted with phosphate-buffered saline in a volume equal to the sputum plus dithiothreitol. Total cell counts were performed on a hemocytometer using Trypan blue stain. Slides were prepared by cytospin (Thermo Fisher Scientific Inc) and were stained with May-Grunwald and Giemsa for differential cell counts. Cell counting was performed by an observer blind to the clinical characteristics of the subjects. At least 500 inflammatory cells were counted in each sample. A sample was considered adequate when the patient was able to expectorate at least 2 mL of sputum and the slides contained < 10% squamous cells on differential cell counting. The total cell count (expressed as the number of cells $\times 10^6$) and the percentage of sputum inflammatory cells was used for analysis. Sputum supernatants were kept at −70°C for further measurement of OPN, transforming growth factor (TGF)-β_1, MMP-2, IL-8, and leukotriene B4 (LTB4) (e-Appendix 1).

Lung Function

FEV_1, FVC, FEV_1/FVC ratio, static volumes (functional residual capacity), and diffusing capacity of the lung for carbon monoxide (DLCO) corrected for hemoglobin levels were measured using a commercially available system (MasterScreen Body; CareFusion Corporation), according to the American Thoracic Society guidelines.[10,11]

Mediator Assays

MMP-2 (free) and IL-8 were measured using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Inc) with detection limits of 3.5 pg/mL and 0.047 ng/mL, respectively. LTB4 was measured with an ELISA kit (Cayman Chemical Company). TGF-β1 and human OPN were measured by ELISA kits (R&D Systems, Inc) with lower detection limits of 50 pg/mL and 6 pg/mL, respectively. All values were expressed as pg/mL. The intraassay and interassay variability were assessed according to the manufacturers' instructions for all the mediators measured and were within acceptable coefficient of variation (%). For OPN, the intraassay and interassay variabilities were 4% and 6.5%, respectively. The recovery and linearity of the assays after appropriate spiking

experiments produced samples with values within the dynamic range of the assay.

Quantification of Emphysema in Patients With COPD

All patients with COPD were submitted to HRCT scan of the chest using a Philips Brilliance 64 (Koninklijke Philips N.V.). The degree of emphysema was calculated by an experienced radiologist using a visual emphysema score as previously described.[12] The presence of emphysematous lesions involving $>15\%$ of the pulmonary parenchyma were used for the characterization of the emphysematous COPD phenotype, as previously described.[13]

Statistical Analysis

Normally distributed data are presented as mean $\pm$ SD, and skewed data are presented as median (interquartile range). Normality of distribution was checked with Kolmogorov-Smirnov test. Statistical comparisons between groups were performed with one way analysis of variance for normally distributed data and Kruskal-Wallis tests for skewed data, accompanied by appropriate post hoc tests for multiple comparisons (Bonferroni and Dunnett, respectively). Differences in numerical variables between two groups were evaluated with unpaired *t* tests or Mann-Whitney *U* tests for normally distributed and skewed data, respectively, whereas comparisons of proportions were performed using χ^2 tests. To examine the associations between OPN, sputum cells, mediators (IL-8, TGF-β_1, MMP-2, LTB4), lung function tests, and emphysema score, linear regression analysis was performed using OPN as the dependent variable, after proper adjustments for age, sex, BMI, smoking habit (in pack-years), Charlson comorbidity index score, duration of the disease, and treatment regimens. Data were interpreted as standardized coefficients with 95% CIs. *P* values $<.05$ (two-sided) were considered statistically significant. Statistical analysis was performed using SPSS 16.0 (IBM) and GraphPad Prism 5 (GraphPad Software, Inc).

Results

Demographic Characteristics

Figure 1 presents the flowchart of patients with COPD included in the study. Seventy-seven patients with COPD and 40 healthy subjects (20 smokers) were included in the study. Forty-one patients with COPD were receiving 1,000 μg fluticasone/d or equivalent. Forty-nine patients were receiving long-acting β_2-agonists, and 61 were receiving tiotropium. Sputum induction was well tolerated by all patients. The demographic characteristics of the study population are summarized in Table 1.

Inflammatory Variables in Induced Sputum

Patients' inflammatory variables are summarized in Table 2. OPN levels (pg/mL) were significantly higher in patients with COPD compared with both smoking and nonsmoking healthy subjects (median [interquartile range], 1,340 [601, 6,227] vs 101 [77, 110] vs 68 [50, 89] pg/mL, respectively; $P<.001$). Moreover, healthy smokers had significantly higher OPN levels compared with healthy nonsmokers (Fig 2, Table 2). A significant difference in the levels of sputum OPN was observed between patients with and without significant emphysema on HRCT scan (4,325 [2,051, 12,236] vs 654 [360, 876] pg/mL; $P<.001$), whereas no difference was observed between patients with and without severe diffusing capacity limitation, as expressed by $D_{LCO}<40\%$ predicted (1,654 [432, 4,328] vs 1,320 [628, 7,916] pg/mL; $P=.97$). MMP-2, IL-8, TGF-β_1, and LTB4 levels were significantly higher in patients with COPD compared with the other study groups (Table 2).

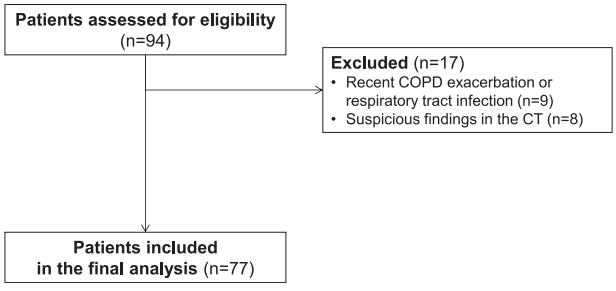

Figure 1 – *Flowchart of patients included in the study.*

Associations of OPN

All the significant associations were observed only in patients with COPD. No association was observed in the healthy smoking and nonsmoking subjects (data not shown).

Inflammatory Biomarkers: Regression analysis after proper adjustments provided significant associations between log OPN levels and sputum neutrophils. Moreover, significant associations were also provided between log OPN levels and IL-8 and MMP-2 (Table 3).

Emphysema Score: Using regression analysis after proper adjustments a significant association was observed between log OPN levels and emphysema score (Table 3).

Lung Function: No significant associations between OPN and lung function tests were observed in any of the study groups (Table 3, for patients with COPD). Stepwise regression analysis showed that MMP-2 and emphysema score represented the strongest associations with OPN ($R^2=0.59$, $P<.001$ and $R^2=0.51$, $P=.001$, respectively).

Discussion

The major finding of the present study is that OPN levels in sputum supernatants are significantly higher in patients with COPD compared with both smoking and nonsmoking healthy subjects. Sputum OPN levels were also positively associated with sputum neutrophils, IL-8, MMP-2, and emphysema score in patients with COPD. This is the first study, to our knowledge, that has evaluated the levels of OPN in the sputum of patients with COPD

TABLE 1] Demographic Characteristics of Study Subjects

Variables	COPD (n = 77)	Healthy Smokers (n = 20)	Healthy Nonsmokers (n = 20)	*P* Value[a]
Age	62 ± 10	60 ± 6	52 ± 10	.623
Sex, female (male)	17 (60)	7 (13)	5 (15)	.425
Smokers, current (former)	36 (41)	13 (7)	0	.540[b]
Duration of the disease, y	7 (5-11)	NA	NA	NA
Smoking, pack-y	70 (58-90)	64 (55-78)	0	.245[b]
BMI, kg/m^2	28 ± 5	27.5 ± 4	27 ± 4	.620
PB FEV_1, % pred	56 (45-69)[c,d]	89 (82-95)	95 (88-99)	< .001
PB FVC, % pred	78 ± 17[c,d]	91 ± 3	96 ± 10	< .001
PB FEV_1/FVC, %	55 ± 11[c,d]	86 ± 7	87 ± 6	< .001
D_{LCO}, % pred	60 ± 16[c,d]	87 ± 7	95 ± 6	< .001
FRC, % pred	112 ± 21[c,d]	89 ± 5	89 ± 12	< .001
Charlson index	5 (2-7)	0	0	…
Treatment regimens		NA	NA	NA
ICS	41			
LABA	49			
Tiotropium	61			
SABA	10			

Normally distributed data are presented as mean ± SD, and skewed data are presented as median (interquartile ranges). *P* values indicate significant differences across the three groups. D_{LCO} = diffusing capacity of the lung for carbon monoxide; FRC = functional residual capacity; ICS = inhaled corticosteroid; LABA = long-acting β_2-agonist; NA = not applicable; PB = postbronchodilation; pred = predicted; SABA = short-acting β_2-agonist.
[a]*P* < .05 considered statistically significant.
[b]Refers to patients with COPD and healthy smokers.
[c]Statistically significant difference compared with healthy smokers.
[d]Statistically significant difference compared with healthy nonsmokers.

and its associations with biomarkers of airway inflammation as well as with the radiologic extent of emphysema, therefore, providing implications for the possible role of OPN in the pathogenesis of COPD.

OPN has been shown to be involved in processes related to smoking habit. A previous study has found that the expression of OPN was highly induced in macrophages from BAL fluid of smokers and that increased expression

TABLE 2] Inflammatory Variables of Study Subjects

Variables	COPD (n = 77)	Healthy Smokers (n = 20)	Healthy Nonsmokers (n = 20)	*P* Value[a]
Cells × 10^6/mL	3.4 (1.5-5.3)[b,c]	0.8 (0.5-1.2)	0.8 (0.6-1.1)	< .001
Eosinophils, %	1 (0-3)	0 (0-0.5)	1 (0-2)	.085
Neutrophils, %	62 (53-75)[b,c]	37 (32-44)[c]	23 (17-25)	< .001
Macrophages, %	35 (24-44)[b,c]	61 (55-67)[c]	72 (58-80)	< .001
Lymphocytes, %	1 (0.5-2)	1 (0-1)	0.25 (0-1)	.550
OPN, pg/mL	1,340 (601-6,227)[b,c]	101 (77-110)[c]	68 (50-89)	< .001
TGF-β_1, pg/mL	1,245 (873-1,609)[b,c]	764 (643-902)	850 (645-1,005)	< .001
IL-8, pg/mL	4,567 (3,411-5,412)[b,c]	899 (823-1,104)[c]	432 (314-556)	< .001
MMP-2, pg/mL	435 (298-670)[b,c]	171 (142-241)[c]	84 (68-102)	< .001
LTB4, pg/mL	190 ± 34[b,c]	107 ± 16	96 ± 22	.003

Normally distributed data are presented as mean ± SD, and skewed data are presented as median (interquartile ranges). *P* values indicate significant differences across the three groups. LTB4 = leukotriene B4; MMP = matrix metalloproteinase; OPN = osteopontin; TGF = transforming growth factor.
[a]*P* < .05 considered statistically significant.
[b]Statistically significant difference compared with healthy smokers.
[c]Statistically significant difference compared with healthy nonsmokers.

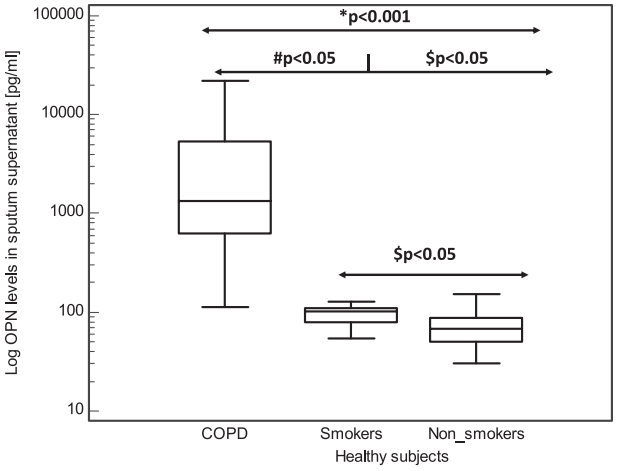

Figure 2 – *OPN values in log scale (pg/mL) in sputum supernatant of patients with COPD and healthy subjects (smokers and nonsmokers).* *P *values indicate significant differences across the three groups. #Statistically significant difference compared with healthy smokers; $Statistically significant difference compared with healthy nonsmokers. OPN = osteopontin.*

levels correlated with lung function impairment in this study group.[14] Moreover, Prasse et al,[15] assessed the essential role of OPN in smoking-related interstitial lung diseases, showing that BAL cells from patients with pulmonary Langerhans cell histiocytosis or desquamative interstitial pneumonitis spontaneously produced abundant amounts of OPN. In the same study, BAL cells from healthy smokers produced 15-fold less OPN, and those from nonsmoking healthy volunteers produced no OPN, suggesting a possible pathogenic role of OPN in both pulmonary Langerhans cell histiocytosis and desquamative interstitial pneumonitis. In a previous study of our group, sputum OPN was higher in smoking compared with nonsmoking subjects with asthma, and it was positively associated with sputum neutrophils, TGF-β_1, and IL-8, suggesting a possible role for OPN in the neutrophilic inflammation and the remodelling process in patients with asthma who smoke.[5]

OPN levels have also been associated with alveolar airspace enlargement, as shown by Schneider et al[4] in a model of COPD $Ada^{-/-}$ double-knockout mice. In that study, $Ada^{-/-}$ mice exhibited OPN-dependent neutrophilia, alveolar airspace enlargement, and increases in mediators of airspace enlargement, such as MMP-9 and its corresponding tissue inhibitor TIMP-1. The authors of this study additionally showed that patients with COPD had increased OPN expression within distal airways that was associated with clinically relevant airway obstruction.[4] Although we did not find any correlation between OPN levels and lung function parameters, our results are compatible with the previously defined findings toward the direction of a possible role of OPN in the induction of neutrophilic inflammation and the pathogenesis of emphysema in COPD.

Conflicting data exist regarding the interaction between OPN and TGF-β_1, as it has been shown that OPN is a TGF-β_1 response gene but also that it may function

TABLE 3] Regression Analysis Between OPN, Inflammatory Cells, Mediators, Lung Function Tests, and Emphysema Score in Patients With COPD

Variables	β Standardized Coefficient (95% CI)	Adjusted R^2	*P* Value[a]
Total cells × 10^6/mL	0.071 (−190, 559)	0.008	.327
Eosinophils, %	−0.098 (−784, 182)	0.037	.217
Neutrophils, %	0.182 (0.2, 4.8)	0.185	.038
Macrophages, %	0.087 (−64, 145)	0.021	.440
Lymphocytes, %	0.009 (−466, 583)	0.009	.743
MMP-2, pg/mL	0.654 (13, 27)	0.590	< .001
IL-8, pg/mL	0.297 (0.07, 0.3)	0.089	.028
TGF-β_1, pg/mL	0.162 (−0.07, 5)	0.038	.060
LTB4, pg/mL	−0.48 (−37, 19)	0.006	.535
Emphysema score	0.311 (741, 2,610)	0.505	.001
FEV_1, % pred	0.075 (−90, 369)	−0.044	.226
FEV_1/FVC, % pred	0.022 (−38, 57)	−0.031	.683
FRC, % pred	−0.032 (−201, 129)	0.004	.661
D_{LCO}, % pred	−0.114 (−0.06, 4.5)	−0.090	.059

Regression analysis was performed after proper adjustments for age, sex, BMI, smoking habit (current or former smokers and pack-y), and treatment regimens. See Table 1 and 2 legends for expansion of abbreviations.
[a]$P < .05$ considered statistically significant.

upstream of TGF-β_1 by regulating its activation in lung fibrosis. [16,17] Our study failed to reveal an association between TGF-β_1 and OPN. On the other hand, it has been shown that TGF-β induces the secretion and activation of the proteolytic enzyme MMP-2, and, conversely, TGF-β can also be activated by a number of proteases, including MMP-2.[18,19] Moreover, in another study it was suggested that TGF-β_1 signaling may prevent emphysema development by inhibiting MMP-mediated elastin degradation or by promoting synthesis of elastin.[20] MMP-2 expression has been found to be elevated in the lung parenchyma of patients with COPD compared with smoking and nonsmoking healthy subjects, and such an upregulation has been related to disease severity, implying a possible role of MMP-2 in airway remodeling in COPD.[21,22] Additionally, other studies have demonstrated increased gelatinolytic activity linked to sputum MMP-2 and a negative correlation of induced sputum MMP-2 with airway obstruction in patients with COPD.[23,24] In accordance with the described studies, our results showed higher induced sputum MMP-2 levels in patients with COPD compared with the other two groups. Moreover, Berman et al[17] showed that MMP-2 expression may be regulated by OPN during lung injury in vivo in a model of murine bleomycin-induced lung fibrosis. In accordance with these findings, our data suggest a role of OPN through MMP-2 in the inflammatory process in the lungs of patients with COPD. However, since the activation of MMP-2 is a complex process, a more detailed evaluation including measurement of the levels of tissue inhibitor of metalloproteinase-2 might be necessary to extract safe conclusions.

An interesting finding of the present study is the correlation of OPN levels to the extent of emphysema, as defined by the emphysema score calculated in HRCT scans. The explanation of such a relation between a measurable marker in induced sputum supernatant and a score yielded from a radiologic evaluation requires caution. However, this association of OPN with emphysema may be mediated through MMP-2, a cytokine we also found to be associated with OPN. Moreover, a study demonstrated that the gene for OPN was highly expressed in the lung antigen-presenting cells of smoke-exposed mice and was required for the development of emphysema in vivo through T(H)17 inflammatory responses, thus, providing a possible mechanism for the induction of emphysema.[25] However, it has to be stressed that the association between OPN and parenchymal destruction is rather speculative, although the fact that it was present in the detailed regression analysis after correction for all possible confounders is supportive of this relationship.

The extremely low OPN levels in both smoking and nonsmoking healthy subjects may be attributed to the absence and/or inactivation of OPN-producing cells. In the present study, OPN was positively associated with sputum neutrophils and IL-8. The association of OPN with sputum neutrophils observed in patients with COPD is compatible with neutrophilic airway inflammation as a major factor in the pathogenesis of the disease.[26] Moreover, the association of OPN with IL-8, which is the major chemoattractant for neutrophils, provides an explanation for the trafficking of neutrophils in the airways and the sputum of patients with COPD. In a previous study in an experimental model of colitis, defective recruitment of neutrophils was observed in OPN-null mice, whereas the addition of exogenous OPN was associated with a robust increase in peritoneal infiltration of neutrophils.[27] Accordingly, OPN could also be directly associated with neutrophilic chemotaxis apart from IL-8. These findings support the importance of OPN in the recruitment of neutrophils and that OPN is a relevant mediator of neutrophil chemotaxis.

The absence of correlation between OPN and lung function points toward a lack of association of OPN with airway obstruction severity. However, this may be attributed to the fact that the number of patients with COPD evaluated is rather small to draw safe conclusions about the different disease stages according to airway obstruction. Additionally, airflow limitation as expressed by FEV_1, is the composite terminal effect of various pathogenic mechanisms in COPD, including inflammation, remodeling, and fibrosis. Previous studies have shown associations between the degree of airflow obstruction and OPN expression in alveolar macrophages of smokers[14] and OPN expression in human surgical lung tissue samples from patients with COPD.[4] Differences in sampling material—induced sputum vs BAL and lung biopsies—may account for the discrepancy of our findings with the previously discussed studies. Interestingly, of all the associations with lung function parameters, the only one presenting a trend toward statistical significance with sputum OPN levels was D_{LCO} ($P = .059$). Despite the fact that the association between D_{LCO} and the extent of emphysema is rather weak, this finding may further support the association between OPN and the extent of parenchymal

destruction in patients with COPD. Furthermore, it has been reported that any associations between DLCO and inflammatory biomarkers in patients with predominant emphysema are mainly related to the inflammatory phenotype observed in small airways.[28]

This study presents limitations. First, the cross-sectional design does not allow us to provide long-term follow-up data of the patients. A prospective cohort study evaluating the importance of sputum OPN as a predictive biomarker of clinically relevant outcomes (eg, exacerbations, hospitalizations, and/or mortality) should be specifically designed for that purpose. Second, the absence of biopsy specimens did not allow us to investigate structural changes in the airways of patients with COPD and their associations with OPN levels. However, the fact that sputum OPN presented significant associations with emphysema score and particular sputum biomarkers related to inflammation and remodeling, which remained even after proper adjustments for confounders, provides important evidence about the possible role of OPN in these processes. Finally, the results of our cross-sectional study cannot rule out the possibility that OPN is not causal or pathophysiologically related to COPD but rather a surrogate marker of inflammation, specific to neither obstructive disease nor neutrophilic inflammation.

Conclusions

OPN levels are significantly elevated in the induced sputum of patients with COPD, compared with smoking and nonsmoking healthy control subjects. The associations of OPN with sputum neutrophils, IL-8, MMP-2, and emphysema score in patients with COPD suggest a possible role for OPN in the induction of neutrophilic inflammation and the pathogenesis of emphysema in COPD. Given the possible implication of OPN in the regulation of multiple processes that promote the pathogenesis of COPD, our findings suggest that OPN may represent an attractive novel biomarker that may serve as a possible therapeutic target.

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Other contributions: This work was performed at the 1st and 2nd Respiratory Medicine University Departments, University of Athens, Medical School, Athens, Greece.

Additional information: The e-Appendix can be found in the Supplemental Materials section of the online article.

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