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Systemic and Lung Inflammation in 2 Phenotypes of Chronic Obstructive Pulmonary Disease

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OBJECTIVE: To study whether patients with chronic obstructive pulmonary disease (COPD) at the same level of flow limitation but with different clinical phenotypes present different degrees of systemic and/or pulmonary inflammation.

PATIENTS AND METHODS: We studied 15 male smokers without COPD (control group) and 39 males with COPD in stable clinical condition.

The COPD patients were assigned to 2 groups based on the ratio of carbon monoxide diffusing capacity (DLCO) to alveolar volume (DLCO/VA) expressed as a percentage as follows: *a)* mainly emphysema (n=15) and *b)* mainly chronic bronchitis (n=24). Classification was determined by comparing both clinical features and diagnostic images.

RESULTS: Mean (SD) concentrations of interleukin 8 (IL-8) and 8-isoprostane in exhaled breath condensate (EBC) were significantly lower in patients with mainly emphysema (IL-8, 0.34 [0.70] pg/mL; 8-isoprostane, 0.07 [0.26] pg/mL) than in patients with chronic bronchitis (IL-8, 2.32 [3.10] pg/mL; 8-isoprostane, 1.77 [2.98] pg/mL) or in the controls (IL-8, 3.14 [4.59] pg/mL; 8-isoprostane, 1.92 [2.84] pg/mL); *P*<.05 for IL-8 comparisons and *P*<.01 for 8-isoprostane.

IL-8, leukotriene B_4, and 8-isoprostano in EBC correlated significantly with DLCO/VA (% of predicted) (*r*=0.30, *P*<.05; *r*=0.29, *P*=<.05; and *r*=0.46, *P*<.01, respectively) but not with forced expiratory volume in 1 second. There was a negative correlation between EBC and serum levels of both IL-8 (*r*=–0.31; *P*<.05) and 8-isoprostane (*r*=–0.51; *P*<.001). The correlation between leukotriene B_4 concentrations in EBC and serum was not significant, however.

No significant differences were found between smokers' and ex-smokers' serum levels of IL-8, leukotriene B_4, 8-isoprostane in serum or EBC.

CONCLUSIONS: The results indicate that COPD patients with an emphysematous phenotype have a less intense inflammatory response and less oxidative stress in the lung.

Key words: *COPD. Phenotype. Inflammation. Oxidative stress.*

Inflamación pulmonar y sistémica en 2 fenotipos de EPOC

OBJETIVO: Investigar si los pacientes con enfermedad pulmonar obstructiva crónica (EPOC) con un mismo grado de limitación ventilatoria, pero diferente fenotipo clínico, presentan diferencias en el grado de respuesta inflamatoria pulmonar y/o sistémica.

PACIENTES Y MÉTODOS: Se estudió a 15 varones fumadores sin EPOC (grupo control) y a 39 varones con EPOC en situación clínica estable. Usando la relación factor de transferencia de monóxido de carbono/volumen alveolar (TLCO/VA%), se dividió a los pacientes con EPOC en 2 grupos: *a)* EPOC de predominio enfisema (EPOC-A; n = 15), y *b)* EPOC de predominio bronquitis crónica (EPOC-B; n = 24). La correcta clasificación de los pacientes se confirmó analizando aspectos clínicos y técnicas de imagen.

RESULTADOS: Las concentraciones medias ± DE de interleucina-8 (IL-8) y de 8-isoprostano en el condensado de aire exhalado (CAE) fueron significativamente menores (p < 0,05 para la IL-8 y p < 0,01 para el 8-isoprostano) en los pacientes con predominio enfisematoso (IL-8: 0,34 ± 0,70 pg/ml; 8-isoprostano: 0,07 ± 0,26 pg/ml) que en los pacientes con bronquitis crónica (IL-8: 2,32 ± 3,10 pg/ml; 8-isoprostano: 1,77 ± 2,98 pg/ml) o que en los controles (IL-8: 3,14 ± 4,59 pg/ml; 8-isoprostano: 1,92 ± 2,84 pg/ml). Los valores de IL-8, leucotrieno B_4 y 8-isoprostano en el CAE se relacionaron significativamente con los valores de TLCO/VA% (r = 0,30, p < 0,05; r = 0,29, p = < 0,05, y r = 0,46; p < 0,01, respectivamente), pero no con el volumen espiratorio forzado en el primer segundo. Existió una relación negativa entre los valores de IL-8 (r = –0,31; p < 0,05) y 8-isoprostano (r = –0,51; p < 0,001) en suero y CAE. Sin embargo, esta correlación no fue significativa para el leucotrieno B_4. No se observaron diferencias significativas entre fumadores activos y ex fumadores para IL-8, leucotrieno B_4 y 8-isoprostano en suero y CAE.

CONCLUSIONES: Los resultados de este estudio indican que en pacientes con EPOC la presencia de un fenotipo enfisematoso se acompaña de una menor respuesta inflamatoria y menor estrés oxidativo en el pulmón.

Palabras clave: *EPOC. Fenotipo. Inflamación. Estrés oxidativo.*

Introduction

Chronic obstructive pulmonary disease (COPD) is an inflammatory process in the lung that can be accompanied by systemic manifestions.[1] One of the most widely accepted hypotheses to explain the

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pathogenesis of COPD is that inflammatory response and oxidative stress are the main causes of change in patients' airways and lungs. An obstacle to overcome when approaching the study of COPD, however, is the heterogeneity of the disease process.[2] COPD brings together a series of processes that range from the ambiguous "asthmatic bronchitis" to bullous emphysema. Logically, differences in the underlying morphology of lesions should lead to clinical and functional variation; moreover, such differences will also affect clinical prognosis. Clinical disparity can be confirmed easily in daily practice, where we can find very different responses to therapy and clinical course for any given degree of airflow limitation.

To date, most studies of markers of inflammation and oxidative stress in COPD have analyzed the results for all types of patients together, even though different degrees of airway, lung, or systemic involvement are present. These differences may explain why patients with the same level of airflow obstruction have marked differences in clinical status, functional decline, and comorbidity.

The most typical phenotypes—emphysema (type A) or chronic bronchitis (type B)—are seen often even though the degree of overlap in many patients makes it difficult to establish cutoff points for guiding treatment. Demonstrating differences between the 2 types may be relevant, however. Even though patients may have mixed clinical forms, the distinction can help us design new management approaches or lead to better understanding of the variability we observe.

The aim of this study was to investigate whether COPD patients with the same degree of airflow limitation but different clinical phenotypes have different degrees of pulmonary or systemic inflammatory responses.

Patients and Methods

Patients

We studied 15 male smokers without COPD (control group) and 39 males with COPD in stable clinical condition. Both controls and patients had smoked at least 20 pack-years and patients had not experienced an exacerbation in the 3 months before the study. COPD was defined according to the criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD).[3] All patients with COPD had received inhaled corticosteroids regularly for 3 months before the study (250 g of fluticasone or 400 g of budesonide every 12 hours). All patients attended a single visit for clinical assessment in which lung function tests were performed. Basic laboratory tests and highly sensitive C-reactive protein and antinuclear antibody assays were performed. Exhaled breath condensate (EBC) was collected. Patients with other pulmonary or systemic diseases were excluded.

Based on the ratio of carbon monoxide diffusing capacity (DLCO) to alveolar volume (VA) expressed as a percentage (DLCO/VA%), the COPD patients were grouped as follows: *a)* patients with mainly emphysema if DCLO/VA% was less than 80% of the predicted value (COPD-A, n=15), and *b)* patients with mainly chronic bronchitis if DLCO/VA% was over 80% (COPD-B, n=24). Patient classification was confirmed by analyzing clinical variables and images, among which were computed tomography (CT) images of the thorax. The presence or absence of clinical and radiologic findings of emphysema confirmed the correct placement of patients in each group, but this information was not used as a criterion for assignment nor analyzed quantitatively. Patient characteristics are shown in the Table. The study was approved by the ethics committee of Hospital Universitario de Guadalajara in Spain and informed consent was obtained from all patients.

Lung Function

The Master Lab system (Jaëger, Würzburg, Germany) was used to obtain spirometry parameters, lung volumes, and DCLO. The reference values of the European Coal and Steel Community were used. DLCO results were corrected for hemoglobin level according to the method of Cotes et al.[4]

EBC Collection and Processing

EBC was collected at the same time of day using an Anacon condenser (Biostec, Valencia, Spain). No patient had smoked in the 12 hours before collection. All breathed through the mouth for 15 minutes at tidal volume with nose clips occluding the nostrils. A valve prevented rebreathing and contamination by saliva. All measurements were performed under stable conditions of temperature and humidity. The mean (SD) volume of EBC collected was 2.02 (0.76) mL for controls, 2.42 (1.17) mL for COPD-B patients, and 1.81 (0.97) for COPD-A patients. Aliquots of 200 L were frozen and stored at –70ºC for later analysis. Other samples were freeze dried. Amylase concentrations were undetectable in all samples, ruling out contamination by saliva.

Measurement of Interleukin-8, Leukotriene B4, 8-Isoprostane, and pH

Interleukin-8 (IL-8) was measured by enzyme-linked immunosorbent assay (BD Biosciences, San Diego,

TABLE
Patient Characteristics*

	Controls	COPD-B	COPD-A
Age, y	56 (6)	67 (8)	68 (9)
BMI, kg/m^2	26 (3)	27 (4)	24 (4)
Cigarette pack-years	35 (24)	46 (16)	60 (25)
Dyspnea, MRC scale†	0.06 (0.25)	1.42 (0.72)	2.06 (0.59)
FEV_1, L	3.9 (0.7)	1.48 (0.4)	1.37 (0.52)
FEV_1, %	107 (18)	54 (12)	55 (16)
FVC, L	4.62 (0.7)	2.95 (0.7)	3.0 (0.6)
FVC, %††	104 (13)	83 (14)	92 (17)
TLC, %	99 (14)	103 (4)	113 (18)
RV, %	107 (39)	141 (50)	162 (42)
DLCO, %§	102 (24)	94 (22)	55 (10)
DLCO/VA, %§	113 (18)	115 (25)	61 (10)
Neutrophils, 10^3/mL	4.1 (1.5)	5.1 (1.7)	4.6 (1.1)
Eosinophils, 10^3/mL	1.77 (1.3)	2.0 (1.1)	0.86 (0.6)
White blood cells, 10^3/mL	2.6 (0.8)	2.1 (0.6)	1.9 (0.5)
Immunoglobulin E, U/mL	193 (240)	134 (168)	43 (53)
ANA positive	0	0	0
Active smokers	15/15	10/24	4/15

*Results are expressed as mean (SD) with the exception of ANA and smoking status. BMI indicates body mass index; MRC, Medical Research Council; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; TLC, total lung capacity; RV, residual volume; DLCO, carbon monoxide diffusing capacity; VA, alveolar volume; ANA, antinuclear antibodies; COPD-A and COPD-B, chronic obstructive pulmonary disease that is mainly emphysema or mainly chronic bronchitis, respectively.
†P<.01 between COPD-A and COPD-B. ††P<.05 between COPD-A and COPD-B.
§P<.000 between COPD-A and COPD-B.

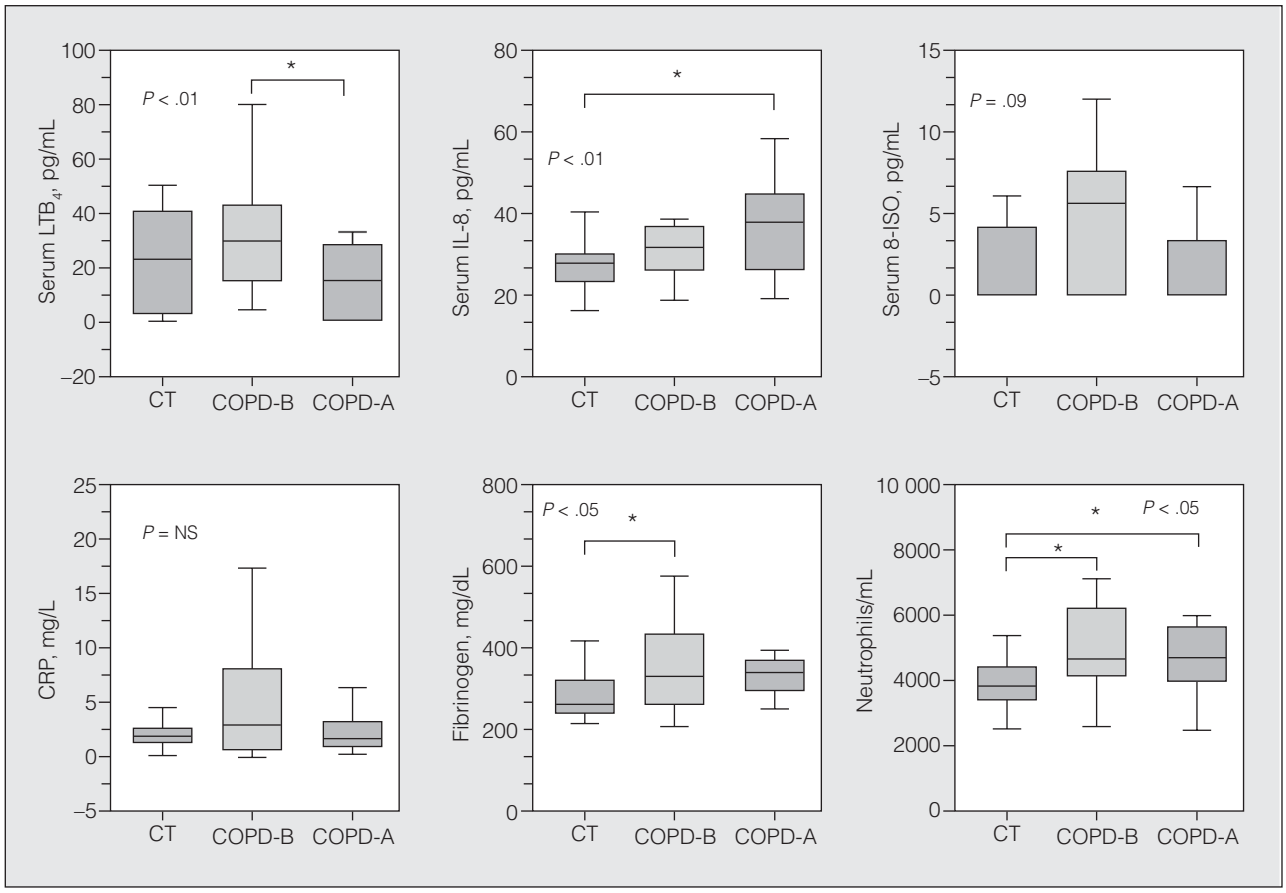

Figure 1. Concentrations of 8-isoprostane (8-ISO) and serum markers of inflammation in the 3 groups studied. Results are expressed as median, interquartile range (box), and upper and lower limits. LTB_4 indicates leukotriene B4; CRP, C-reactive protein; IL-8, interleukin 8; CT, controls; COPD-A and COPD-B, chronic obstructive pulmonary disease that is mainly emphysema or mainly chronic bronchitis, respectively; NS, not significant. *Significant differences, Dunn test.

California, USA) without identification of cross-reactive antigens. Samples were lyophilized and reconstituted to a quarter of their original volume.

Before samples were lyophilized, leukotriene B4 (LTB_4) was quantified with kits (Cayman Chemical Company, Ann Arbor, Michigan, USA) able to detect cross reactivity with other leukotrienes to a limit of 0.01%.

A specific kit (Cayman Chemical Company) was used to analyze 8-isoprostane (8-ISO) as a marker of oxidative stress in the lung.

In all cases, concentrations below the detection limits were recorded as undetectable.

A pH meter (CG 840, Schott Ibérica, Spain) was used to measure the pH of deaerated EBC samples immediately after collection.

Statistical Analysis

Normally distributed data were expressed as means (SD). To compare means, we used analysis of variance and the Student *t* test with Bonferroni correction. When data was not normally distributed nonparametric tests (Kruskal-Wallis and Dunn tests) were applied to detect between-group differences. Spearman's test of correlation was used to detect relations between variables. The level of statistical significance was set at a value of *P* less than .05.

Results

The table and Figure 1 show that neutrophil counts were significantly higher in patients with COPD than in the controls (COPD-A, 4.6 [1.1] 10^3/mL; COPD-B, 5.1 [1.7] 10^3/mL; controls: 4.1 [1.5] 10^3/mL). No differences between the type A and type B COPD groups were found, however. Significantly higher serum levels of IL-8 were observed in patients in the group with mainly emphysema (type A) in comparison with the controls (COPD-A, 29.37 [15.03] pg/mL; COPD-B, 29.17 [32.89] pg/mL; controls, 13.45 [7.78] pg/mL). Fibrinogen levels were higher in the patients with chronic bronchitis (group B) in comparison with controls (COPD-A, 350 [102] mg/dL; COPD-B, 353 [180] mg/dL; controls, 246 [117] mg/dL). The differences between the 2 patient groups, however, did not reach statistical significance for these variables. The differences in 8-ISO and highly sensitive C-reactive protein tests were not statistically significant between the 3 groups. Serum LTB_4 levels were significantly lower in the COPD-A group than in the COPD-B or control groups (Figure 1).

IL-8 and 8-ISO concentrations in EBC were significantly lower in emphysematous patients (IL-8, 0.34 [0.70] pg/mL; 8-ISO, 0.07 [0.26] pg/mL) than in either

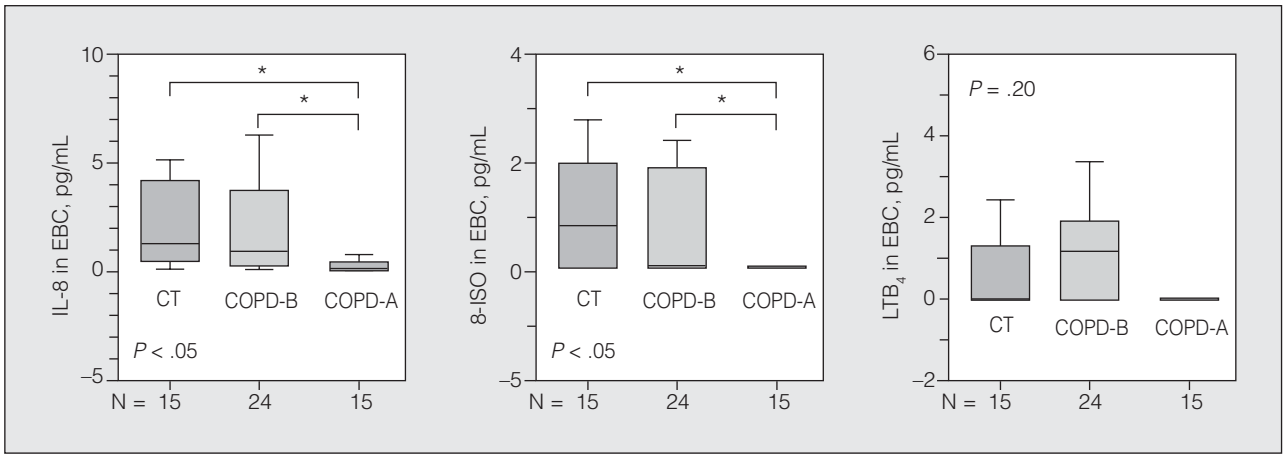

Figure 2. Concentrations of interleukin 8 (IL-8), 8-isoprostane (8-ISO), and leukotriene B4 (LTB_4) in exhaled breath condensate (EBC) for the 3 study groups. Results are expressed as median, interquartile range (box), and upper and lower limits. CT indicates controls; COPD-A and COPD-B, chronic obstructive pulmonary disease that is mainly emphysema or mainly chronic bronchitis, respectively.
***Significant differences, Dunn test.**

those with chronic bronchitis (IL-8, 2.32 [3.10] pg/mL; 8-ISO, 1.77 [2.98] pg/mL) or controls (IL-8, 3.14 [4.59] pg/mL; 8-ISO, 1.92 [2.84] pg/mL); $P<.05$ for IL-8 and $P<.01$ for 8-ISO comparisons. A nonsignificant trend toward lower LTB_4 levels in the COPD-A group was observed ($P=.20$, Figure 2). The pH of EBC samples from COPD patients was significantly lower in comparison with the pH of samples from controls but no difference was found between the 2 phenotypes (Figure 3).

IL-8, LTB_4, and 8-ISO levels in EBC correlated significantly with DLCO/VA% ($r=0.30$, $P<.05$; $r=0.29$, $P=<.05$; and $r=0.46$, $P<.01$, respectively) but not with forced expiratory volume in 1 second (FEV_1). There was a negative correlation between EBC and serum levels of both IL-8 ($r=–0.31$; $P<.05$) and 8-ISO ($r=–0.51$; $P<.05$). No significant relation was observed for LTB_4, however. Nor were there significant differences in IL-8, LTB_4, or 8-ISO serum or EBC levels between active and former smokers.

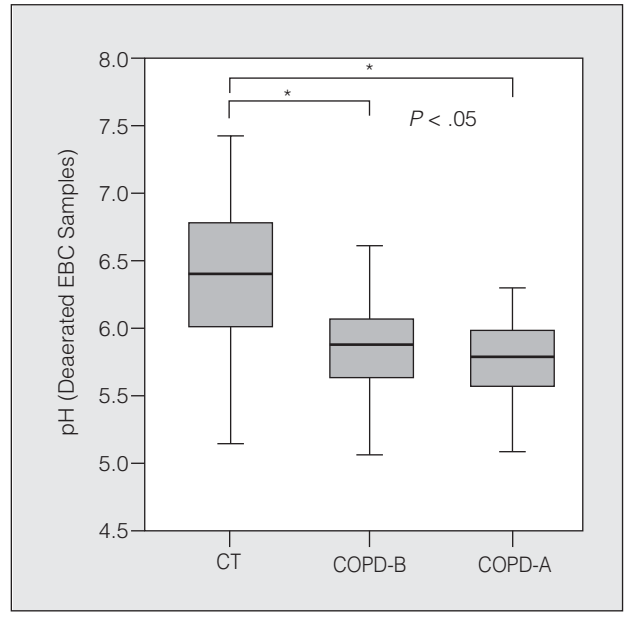

Figure 3. Significantly lower pH values were observed in the exhaled breath condensate (EBC) samples from patients with chronic obstructive pulmonary disease (COPD) in comparison with controls. No significant difference was observed between the COPD phenotypes, however. CT indicates controls; COPD-A and COPD-B, disease that is mainly emphysema or mainly chronic bronchitis, respectively.

Discussion

Two important findings have emerged from this study. First, we saw that in a well-defined population of COPD patients, those with mainly emphysema have lower IL-8 and 8-ISO levels in EBC. Second, patients with COPD have elevated serum levels of IL-8 and other inflammatory markers, but no differences between the 2 phenotypes could be found. On the other hand, patients with emphysematous COPD did have significantly lower LTB_4 serum levels than those with chronic bronchitis or controls. These findings and the lack of a positive correlation between IL-8, LTB_4, and 8-ISO levels in EBC and serum indicate that local and systemic markers of inflammation and oxidative stress may be relatively different.

Clearly, a better understanding of pulmonary and systemic manifestations of COPD will allow us to take a more targeted approach to managing these patients appropriately.

Standard therapy for COPD patients currently includes inhaled medication. In the presence of pulmonary inflammation or oxidative stress, administration of inhaled medication can modify the inflammatory process in both airways and the lung itself. For this reason, it is logical to assume that it may be useful to identify elevated inflammatory and oxidative stress markers in the lung, regardless of FEV_1 values. A better characterization of the different COPD phenotypes would allow us to improve our understanding of why response to treatment or clinical course varies even when FEV_1 values are

similar. This was proposed by Engelen and coworkers,[5,6] who were able to demonstrate significant differences in constitutional characteristics, not only between COPD patients and controls but also between patients with mainly emphysema or mainly chronic bronchitis classified by standard clinical features and chest CT findings.[7]

To date, when inflammatory response or degree of pulmonary oxidative stress has been assessed, the possibility that histologic differences in the lung parenchyma or airways might mean that local and systemic differences might exist has not been taken into account.[8-14] The most important characteristic of pulmonary emphysema is loss of lung parenchyma, blood vessels, and small airways. Therefore, we can speculate that patients with more emphysematous change might have a lower respiratory inflammatory load and less oxidative stress overall and, if such loss is also the cause of serum changes, there would also be a systemic effect. Regardless, some smokers with pulmonary emphysema might develop panacinar lesions that are characterized by low-grade inflammation of the small airways.[2,15]

Recent years have seen great interest develop in analyzing a variety of components collected in EBC as a noninvasive way to study inflammatory response and oxidative stress in the lung.[16] Detecting nonvolatile mediators and inflammatory markers such as IL-8, 8-ISO, LTB_4, and pH can help identify what is happening in the tracheobronchial tree and alveoli. If tracheobronchial events are the origin of these markers, loss of lung parenchyma, vessels, and small airways should correlate with a decrease in mediators in EBC in this subgroup of COPD patients.

Our pilot study suggests that for the same degree of airflow limitation, subjects with a mainly emphysematous phenotype have higher titers of inflammatory markers in EBC. Clinically, these findings might explain in part why patients with mainly emphysematous COPD have a lower functional response to treatment.[17-19]

Differences were much less evident in serum markers. The mechanisms that participate in systemic inflammation in COPD patients are currently unknown. In fact, many mechanisms may be involved. If pulmonary changes are the source of systemic inflammation, any intervention such as inhaled corticosteroid therapy that is able to reduce inflammatory response in the lung might be useful systemically as well. In fact, this hypothesis has recently been proposed to explain the reduction in cardiac events in COPD patients taking inhaled corticosteroids. Systemic alterations in COPD might also be directly induced by tobacco smoke. However, we found no significant differences in IL-8, LTB_4, or 8-ISO in serum and EBC between active and former smokers, and this would suggest that these markers reflect an inflammatory response, not the direct action of tobacco smoke. Finally, a third possibility is that some of the alterations described in circulating inflammatory markers are the cause, not the consequence of COPD.[7] Our data demonstrate some of the differences in systemic markers between smokers in the control group and the COPD phenotypes. Those differences were much more marked in EBC, however. This indicates that local and systemic response may have specific characteristics that are relatively independent of tobacco smoke.

The use of inhaled corticosteroids in COPD patients can interfere with the results of some serum mediators[20]; the patient groups in our study were homogeneous in that respect, however. The impact of this therapy on EBC mediators, on the other hand, is minimal.[21]

An important limitation of our study was that no well-established cutoffs are currently available to distinguish the 2 COPD phenotypes. In fact, a large percentage of patients with COPD may overlap to a large degree. We used CT images and clinical characteristics to confirm the classification of patients by phenotype, but the main criterion for distinguishing the groups was the presence of a low DLCO/VA%. The studies that have found a relationship between macroscopic emphysematous lesions and a variety of lung function tests to date have reported weak correlations and discrepancies are considerable. However, McLean and co-workers[22] reported that diffusion expressed as a coefficient derived from its ratio to VA (DCLO/VA) had good correlation with alveolar surface per unit of volume. This ratio was constant regardless of the presence of macroscopic emphysematous lesions or type or severity of radiologic emphysema. Given that this test is unaffected by the disordered distribution of ventilation in patients with severe airflow obstruction, a finding of low DLCO/VA% in the absence of other diseases reflects the degree of emphysema, regardless of whether there might be associated airway abnormalities.[22,23] In fact, the correlations between markers in EBC and DLCO/VA% but not FEV_1% suggest that for the same degree of airflow obstruction the presence of pulmonary emphysema affects the level of oxidative stress and lung inflammation in this disease. We are inclined to use DLCO/VA% as a screening criterion, given the simplicity of the diffusion test, its reliability, its contribution to the overall assessment of changes in the lung, and the difficulties of standardizing quantitative assessment of CT findings reliably. We use CT and clinical features as qualitative contributions for confirmation.

Another limitation of our study was that in some cases tests were insufficiently sensitive and, in general terms, markers in EBC could not be assessed reliably.[24,25]

Nevertheless, in spite of great variability in the technique used, we have made an effort to control for technical aspects during all phases of analysis.[26] Furthermore, the consistency of our results, showing clear differences between the 2 COPD phenotypes, suggests that they can not be explained by technical variables that might have interfered with the analysis of EBC samples.

In summary, findings from this study indicate that in a homogeneous population of COPD patients with mainly emphysema, the degree of pulmonary inflammation and oxidative stress will be reduced. Although in clinical practice many cases present overlapping features of both

phenotypes and may not be clearly distinguishable, these findings should be borne in mind because they may be relevant to the interpretation of pathogenesis and to the treatment of individual patients.

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