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INFLAMMATION, OXIDATIVE STRESS AND SYSTEMIC EFFECTS IN MILD CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Chronic obstructive pulmonary diseases (COPD) is a pulmonary disease characterized by systemic abnormalities. The aim of this study is to investigate inflammation and systemic effects in mild COPD. Twenty-seven mild stable smoking related COPD patients and 15 age-matched healthy subjects were enrolled in the study. IL-6, TNF-α and IL-4 in plasma, sputum and exhaled breath condensate were measured. We also measured exhaled nitric oxide (NO) and pH in sputum and in breath condensate. Moreover, fat-free mass, body mass index (BMI), respiratory muscle strength, plasma oxidative stress and C-reactive protein (CRP) were measured. Higher concentrations were found of CRP, of diacron reactive oxygen metabolites (DROMs) and of IL-6, TNF-α and IL-4 either in plasma or in supernatant of induced sputum or in exhaled breath condensate of COPD subjects compared to healthy controls. Furthermore, higher concentrations were observed of exhaled NO and lower exhaled pH in breath condensate of COPD when compared with healthy subjects. In the group of COPD patients, the subjects with airway reversibility showed an increase of sputum eosinophils and exhaled NO, whereas the subjects without airway obstruction reversibility showed an increase in sputum neutrophils, TNF-α and IL-6. We also found a trend towards a decrease in fat-free mass and respiratory muscle strength in COPD compared to healthy subjects and a negative correlation between these systemic indices (fat-free mass, maximal inspiratory pressure, maximal expiratory pressure) and TNF-α concentrations in the blood, sputum and breath condensate. We conclude that mild COPD subjects present an increase in inflammatory markers in blood and in airways of similar pattern and furthermore, the neutrophilic pattern of airway inflammation observed in the group of COPD subjects without an airway obstruction reversibility makes it more likely that systemic features are present.

Chronic obstructive pulmonary disease (COPD) is usually considered to be a pulmonary disease (1). However, recent studies have demonstrated several extra-pulmonary abnormalities, particularly in more severely affected patients, that suggest the necessity of a wider definition of this disease (2). Several systemic features have been described in COPD patients, including systemic inflammation and oxidative stress, skeletal muscle dysfunction (SMD), weight loss, atherosclerosis and osteoporosis (3-5).

Key words: COPD, bronchodilator reversibility, systemic effects, inflammation, oxidative stress

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These systemic effects may have an adverse effect on the quality of life and may contribute towards the mortality of COPD (6-8). Systemic inflammation and oxidative stress appear to be risk factors for the majority of the systemic complications in COPD patients (9). Several hypotheses have been suggested to explain how pulmonary inflammation and oxidative stress may spill over into the systemic circulation (10-11). There may be a genetic predisposition in some COPD subjects to develop both systemic and pulmonary inflammation (12-13). However, to date the exact mechanisms through which pulmonary inflammation and oxidative stress result in systemic effects in COPD remain unknown (2, 9).

Moreover, increased reversibility to bronchodilators in COPD patients may share some of the features of asthma, such as eosinophilia (14-16) and increased exhaled nitric oxide (NO) (17). Recently, our group demonstrated that COPD subjects with reversible obstruction present lower oxidative stress in airways (18). These more reversible COPD patients may represent an intermediate group of patients between asthma and non-reversible COPD or may be COPD patients who also have concomitant asthma (19).

The aim of this study is to investigate airway inflammation and systemic effects in mild COPD when compared with a group of healthy subjects. In particular we measured interleukin (IL)-6, tumor necrosis factor (TNF)-α and interleukin (IL)-4 in plasma, induced sputum and breath condensate, exhaled nitric oxide (NO), exhaled pH, inflammatory sputum cells and plasmatic C reactive protein (PCR) to investigate airway and sistemic inflammation and fat-free mass, body mass index (BMI), respiratory muscle strength, plasma oxidative stress to study the systemic effects of COPD.

MATERIALS AND METHODS

Study population

Twenty seven mild stable ex-smoker COPD (52±4 yrs, 21 M, BASAL FEV_1 1.9±0.4 lt, 75.3±3.1% of predicted) and 15 healthy ex-smoker subjects (49±7 yrs, 12 M, BASAL FEV_1 2.6±0.6 lt, 110.4±6.1% of predicted) with no history of lung disease were studied (Table I). The diagnosis of COPD was based on ERS/ATS Guidelines (1). All subjects were recruited from the Respiratory Disease Institute, University of Foggia, and written informed consent was obtained from all subjects. The study was approved by the Institutional Ethics Committee.

None of the COPD patients had a history of asthma or atopy or positive skin prick test, or were suffering from chronic congestive heart failure. All patients were in a stable condition at the time of the study and free from acute exacerbations of symptoms or upper respiratory tract infections in the 3 months preceding the study. Only mild COPD were selected to avoid the influence on systemic effects of overlapped conditions that often appear in advanced stages of COPD (cardiovasura disorders, respiratory failure and others).

Healthy subjects had no respiratory symptoms and no respiratory tract infection for ≥3 months prior to the study. All COPD patients and healthy subjects enrolled were ex-smokers from at least 6 months preceding the study. COPD patients were enrolled during routine hospital visits. A full medical history was taken and general physical examination was performed. Patients then underwent lung function testing, skin prick test for common inhalant allergens, serum total IgE, arterial blood gas analysis, laboratory tests, fat-free mass, maximal inspiratory pressure (PImax), maximum expiratory pressure (PEmax), exhaled NO measurement, blood and breath condensate and induced sputum collection. None of patients included was treated with oral steroids and only short-acting inhaled β_2-agonists were allowed for symptom relief.

Study design

On the first day subjects underwent clinical data collection, physical analysis, lung function, fat free mass and respiratory muscle strength measurements and blood tests.

On the second day exhaled NO, exhaled breath condensate collection and sputum induction were performed.

Pulmonary function testing and assessment of airflow limitation

Pulmonary function tests were performed prior to the measurement of exhaled breath condensate. FEV_1, FVC and FEV_1/FVC ratio were measured using a dry spirometer (Vmax Spectra series, SensorMedics Corporation, Yorba Linda, California, USA). The highest value of three manoeuvres was expressed as a percentage of the predicted normal value. Reversible airflow limitation were defined as an increase in FEV_1 of ≥200 ml and/or ≥12% from baseline 30 min after inhalation of 200 μg of salbutamol (1).

Fat-free mass measurement

Fat-free mass was determined by bioelectric

impedance analysis (Bodystat 1500; Douglas, UK) (20).

Respiratory muscle strength

Respiratory muscle strength was assessed by measurement of mouth pressure at maximal static inspiratory and espiratory efforts from residual volume and total lung capacity, PImax (maximal inspiratory pressure) and PEmax (maximal expiratory pressure) against an obstructed mouthpiece with a small leak to minimize oral pressure artifacts (Spirovis, Cosmed S.R.L., Italy) (21).

Induced sputum

Sputum was collected and processed according to the method of Spanevello et al (22). All sputum counts and measurements were performed blind to the clinical details. Definition of an adequate selected sputum was one in which there were fewer than 20% squamous cells and viability > 50%. A specific enzyme immunoassay kit (Cayman Chemical, Ann Arbor, Michigan, USA) was used to measure TNF-α and IL-6 concentrations in supernatant of induced sputum.

Exhaled breath condensate and assay

Exhaled breath condensate (EBC) was collected by using the EcoScreen, (Jaeger, Wurzburg, Germany). The subjects breathed through a mouthpiece and a two-way non-rebreathing valve, which also served as a saliva trap. They were asked to breathe at a normal frequency and tidal volume, wearing a nose clip, for a period of 10 min. If subjects felt saliva in their mouth they were instructed to swallow it. Condensate, at least 1 ml, was collected as ice at –20°C, transferred to Eppendorf tubes and immediately stored at -70°C. Samples were analysed within 3 months from collection. To exclude saliva contamination amylase activity was analysed in EBC.

Measurement of exhaled NO

A rapid-response chemiluminescence NO analyzer (model 280; Sievers Instruments; Boulder, Colorado, USA) was used to quantify NO. Two-point calibrations were performed daily using 5.2-parts per million calibration gas. Exhaled NO (F_ENO) was measured using a previously described restricted breathing technique, which employed expiratory resistance and positive mouth pressure to close the velum and exclude nasal NO, and a constant expiratory flow of 45 mL/s. The inhaled gas was ambient air that was passed through a filter to reduce inhaled NO concentrations to <5 parts per billion (ppb). Subjects inhaled to total lung capacity, and exhaled while targeting a constant pressure of 20 mm Hg. Exhalations proceeded until a clear NO plateau of at least 3s duration was achieved. Repeated exhalations were performed until three plateaus agreed within 5% (23).

IL-6, TNF-α and IL-4 measurements

A specific enzyme immunoassay kit (Cayman Chemical, Ann Arbor, Michigan, USA) was used to measure IL-6, TNF-α and IL-4 concentrations in plasma, supernatant of induced sputum and breath condensate. The intra-assay and inter-assay variability were ≤10% for each assay and the detection limit of the assays was 1.5 pg/ml, 4 pg/ml and 20 pg/ml, respectively. The coefficient of variation for IL-6, TNF-α, IL-4 and pH were 5.9%; 3,3%; 4.2% and 0.4% respectively. Reproducibility of exhaled IL-6, TNF-α and pH measurements were assessed in 10 non-smoking normal subjects.

pH measurements

A stable pH was achieved in all cases after deaeration/ decarbonation of supernatant of induced sputum and breath condensate specimens by bubbling with argon (350 ml/min) for 10 min, as previously described (24). pH was then measured within 5 minutes of condensate collection by means of a pH meter (Jenway-350, Ltd Gransmore Green, UK) with a 2 to 16.00 pH range and a resolution/ accuracy on the order of 0.01 ± 0.02 pH.

Plasma reactive oxygen metabolites measurement

Systemic oxidative stress was measured by the diacron reactive oxygen metabolites (D-ROM) test (Diacron International, Italy) (25). Values of DROMS were expressed as Carratelli Units, where 1 U.CARR corresponds to 0.8 mg/L H_2O_2.

Plasma CRP measurement

Plasma CRP was measured using the N high sensitivity CRP Kit (Dade Berhing, Marburg, Germany) which has a lower limit of detection of 0.15 mg/L.

Statistical analysis

Data are expressed as means±SD. A Mann-Whitney test was used to compare groups, and correlations between variables were performed using Spearman's rank correlation test. Significance was defined as a p value of <0.05.

RESULTS

Lung function

All COPD were defined as mild according to GOLD guidelines (26). COPD subjects showed lower percentages of FEV_1 and FEV_1/FVC ratio compared to healthy controls (82.6±1.5 and 65.8±2.2 vs 110.6±7.1 and 81.3±1.4; $p<0.01$ and $p<0.005$)

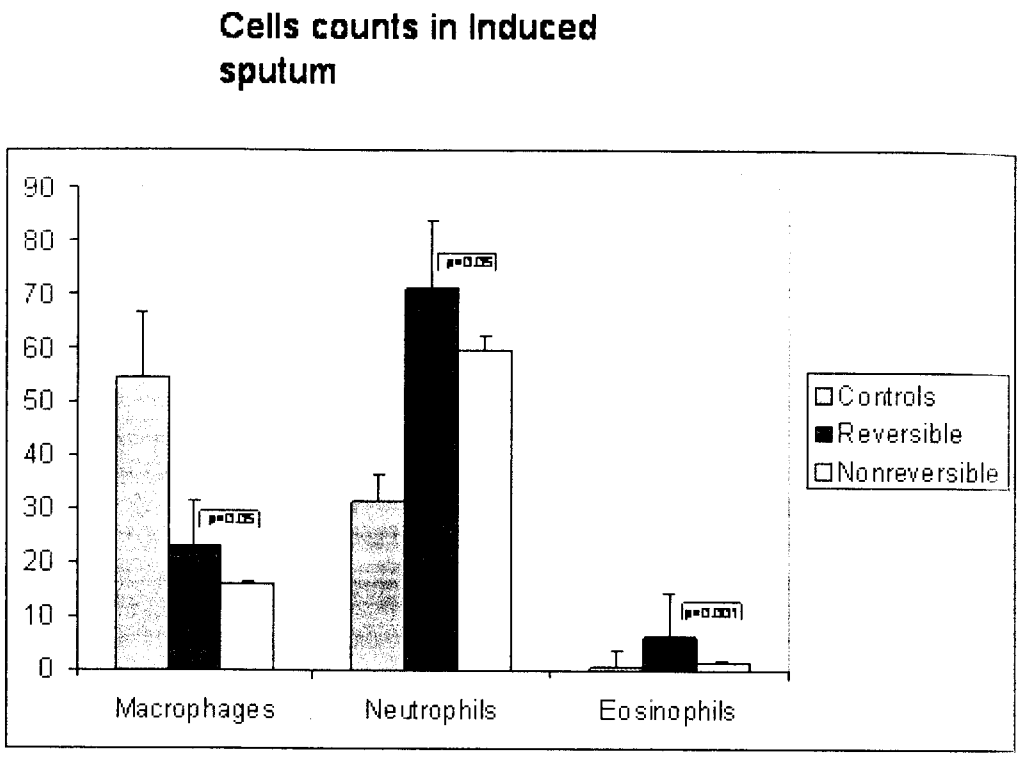

Fig. 1. *Comparison between non-reversible COPD, reversible COPD and healthy controls in terms of percentage of cells found in the induced sputum. We observed a higher percentage of neutrophils in non-reversible COPD than in reversible and of eosinophils in reversible COPD than in non-reversible.*

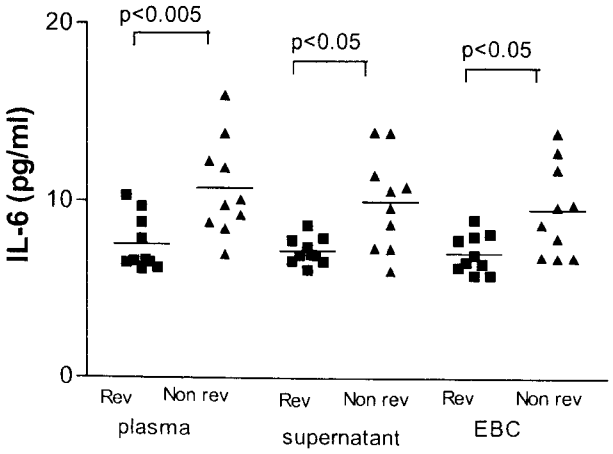

Fig. 2. *Concentrations of IL-6 in COPD subjects and healthy controls in plasma, supernatant of induced sputum and exhaled breath condensate. We found elevated levels IL-6 in supernatant of induced sputum and in exhaled breath condensate of COPD subjects than in healthy controls. Higher concentrations of IL-6 were observed in non-reversible COPD compared to reversible COPD.*

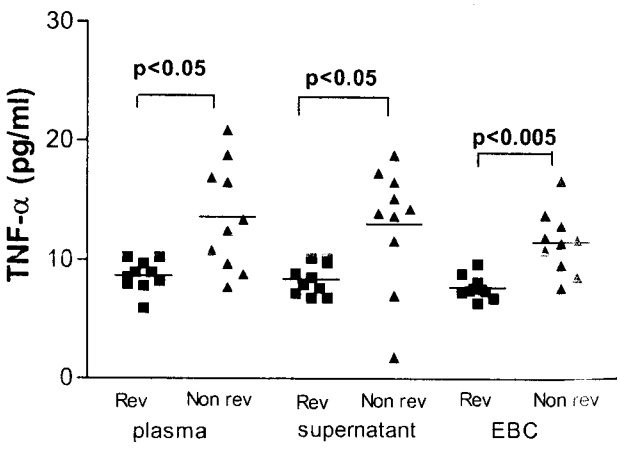

Fig. 3. *Concentrations of TNF-α in COPD subjects and healthy controls in plasma, supernatant of induced sputum and exhaled breath condensate. We found elevated levels TNF-α in supernatant of induced sputum and in exhaled breath condensate of COPD subjects than in healthy controls. Higher concentrations of TNF-α were observed in non-reversible COPD compared to reversible COPD.*

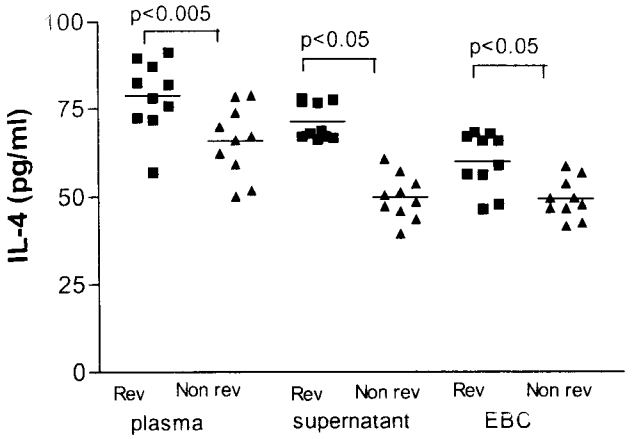

Fig. 4. *Concentrations of IL-4 in COPD subjects and healthy controls in plasma, supernatant of induced sputum and exhaled breath condensate. We found elevated levels IL-4 in supernatant of induced sputum and in exhaled breath condensate of COPD subjects than in healthy controls. Higher concentrations of IL-4 were observed in reversible COPD compared to non-reversible COPD.*

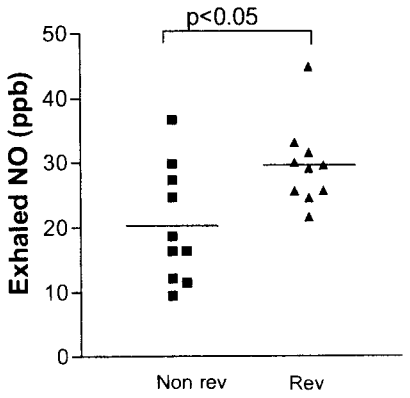

Fig. 5. *Concentrations of exhaled nitric oxide (NO) in COPD subjects and healthy controls. Elevated concentrations of exhaled NO were observed in COPD patients compared to control subjects and in reversible compared to non-reversible COPD.*

(Table I). After reversibility test with salbutamol (400 mcg) required for classification of COPD by guidelines, 10 COPD (mean age 53±3, FEV_1 83.5±1.4, pack/years 30.8±4.9) had a reversibility >12% and 17 had a reversibility <12% (mean age 55±5, FEV_1 85.1±1.5, pack/years 31.0±45.1).

Sputum measurements

Sputum induction was performed in all subjects without observing any significant adverse effect or a decrease in FEV_1 >20%. Four healthy subjects and 6 COPD patients did not produce adequate sputum samples, and their expectorates were discarded. Table II and Fig. 1 show the comparison between the three groups in term of percentage of cells found in the induced sputum.

Higher percentages were found of neutrophils and eosinophils and a reduction of macrophages in COPD subjects (66.4±8.8, 4.6±1.1 and 20.9±4.1%) than in healthy controls (31.3±7.2, 0.5 ±0.3 and 54.7±6.5%)

A higher percentage was observed of neutrophils in non-reversible COPD than in reversible (71.0±9.5 and 59.6±8.6%, $p<0.05$) and of eosinophils in reversible COPD than in non-reversible (6.3±1.1 and 1.5±0.3%, $p<0.05$).

Elevated concentrations of IL-6 (8.54±2.02 vs 5.41±0.87 pg/ml), TNF-α (11.17±3.41 vs 6.22±0.82 pg/ml), IL-4 (60.34±10.66 vs 41.41±5.97 pg/ml) and lower pH (7.50±0.06 vs 7.86±0.18) were found in supernatant of induced sputum of COPD subjects than in healthy controls (Figures 2-4). Higher concentrations of IL-6 (10.39±2.28 vs 7.21±0.73 pg/ml) and TNF-α (14.51±3.32 vs 8.37±1.32 pg/ml) were observed in supernatant of sputum of non-reversible and of IL-4 (71.24±5.27 vs 49.94±6.29 pg/ml) of reversible COPD patients (Figures 2-4). Lower values of pH were found in non-reversible compared to reversible COPD (7.48±0.07 vs 7.52±0.05).

Exhaled breath condensate measurements

Elevated concentrations of IL-6 (8.30±1.94 vs 4.18±0.63 pg/ml), TNF-α (9.53±2.35 vs 5.65±0.99 pg/ml), IL-4 (55.15±7.88 vs 34.83±5.79 pg/ml) and a lower pH (7.46±0.08 vs 7.68±0.23) were found in exhaled breath condensate of COPD subjects compared to healthy controls (Table I).

Higher concentrations of IL-6 (9.49±2.55 vs 7.08±1.07 pg/ml) and TNF-α (11.51±2.60 vs 7.72±0.68 pg/ml) and lower concentrations of IL-4 (49.49±5.56 vs 60.07±8.13 pg/ml) were observed in exhaled breath condensate of non-reversible compared to reversible COPD (Fig. 2-4). Lower levels of pH were found in exhaled breath condensate of reversible compared to non-reversible

Table I. *Characteristics of COPD and healthy subjects.*

	COPD ex-smokers (n.27)	Healthy subjects ex-smokers (n.15)	p
Sex (M/F)	24/3	12/3	-
Smoke habit (pack/yrs)	30.3±5.0	10.6 ± 3.2	0.001
BMI (kg/m^2)	21.1±2.4	24.5±2.6	ns
FEV_1 (%pred)	82.6±1.6	110.4±6.1	<0.01
FEV_1/FVC %	65.8±2.2	81.3±1.4	<0.005
IgE (kU/L)	13±4	11±7	ns
Skin prick test	negative	negative	
PaO_2 (mmHg)	74.4±3.9	75.4 ± 2.4	ns
$PaCO_2$ (mmHg)	37.9±2.5	40.1 ± 1.7	ns
White blood cells (x10^9/l)	7.9±1.9	5.4±1.2	<0.01
Blood eosinophils (x10^9/l)	0.41± 0.26	0.23±0. 15	<0.01
CRP (mg/l)	11.55 ± 11.11	0.7±0.9	<0.001
Fat-free mass (kg)	46.42 ± 4.49	53.5±8.8	<0.05
PImax (cm H_2O)	-65.55 ± 20.88	- 105.8 ± 2.4	<0.001
PEmax (cm H_2O)	54.78 ± 23.52	192.5 ± 6.7	<0.001
DROM (U.CARR)	267.8 ± 34.12	36.8 ± 6.0	<0.001
Exhaled NO (ppb)	24.97±7.94	9.07±1.64	<0.001
Plasma IL-6 (pg/ml)	9.18±2.31	5.82±0.89	<0.001
Sputum IL-6 (pg/ml)	8.54±2.02	5.41±0.87	<0.001
Condensate IL-6 (pg/ml)	8.30±1.94	4.18±0.63	<0.001
Plasma TNF-α (pg/ml)	11.22 ± 3.67	6.0.6 ± 1.55	<0.001
Sputum TNF-α (pg/ml)	11.17±3.41	6.22±0.82	<0.001
Condensate TNF-α (pg/ml)	9.53±2.35	5.65±0.99	<0.001
Plasma IL-4 (pg/ml)	72.43±10.43	38.71±5.33	<0.001
Sputum IL-4 (pg/ml)	60.34±10.66	41.41±5.97	<0.001
Condensate IL-4 (pg/ml)	55.15±7.88	34.83±5.79	<0.001
Sputum pH	7.50 ± 0.06	7.86 ± 0.18	<0.05
Condensate pH	7.46 ± 0.08	7.68 ± 0.23	<0.05

Data are shown as mean± SD. P= statistical comparison. A Mann-Whitney test was used to compare groups
BMI: body mass index, FEV_1: forced expiratory volume in 1 s, FVC: forced vital capacity, CRP: C-reactive protein, PImax: maximum inspiratory pressure, PEmax: maximum expiratory pressure, DROM: reactive oxygen metabolites, NO: nitric oxide, IL: interleukin, TNF: tumor necrosis factor

Table II. *Total and differential cell counts in induced sputum. Comparison between patients with reversible COPD, patients with non-reversible COPD and healthy subjects in terms of percentage of cells found in the induced sputum. A significantly higher percentage was observed of neutrophils in non-reversible COPD than in reversible and of eosinophils in reversible COPD than in non-reversible.*

	Reversible COPD	Non-reversible COPD	Healthy subjects
Total cell count ($\times 10^6$/ml)	2.9±0.6	3.2±0.8	2.6±0.6
Macrophages (%)	23± 4.9	16±3.2	54.5±6.7
Neutrophils (%)	59.6±8.5*	71.0±9.6**	31.4±7.1
Eosinophils (%)	6.3±1.2**	1.5±0.3	0.5 ±0.2
Lymphocytes (%)	0.9±0.5	2.3±0.5	1.1±0.6
Epithelial cells (%)	9.1±2.4	10.1±4.3	11.1±3.8

*Reversible COPD vs non-reversible COPD: *p <0.05; **p <0.01*

COPD (7.43±0.07 vs 7.49±0.06) (Fig. 2-4). Elevated concentrations of exhaled NO were observed in COPD patients compared to control subjects (24.97±7.94 vs 9.07±1.64 ppb) and in reversible compared to non-reversible COPD (29.59±6.41 vs 20.3±8.9 ppb) (Table I and Fig. 5).

Systemic measurements

A significant reduction of a fat free mass (46.4±24.49 vs 53.5±8.8 kg), of PImax (-65.55±20.88 vs 105.8±2.4 cmH_2O) and PEmax (54.78±23.52 vs 192.5±6.7 cmH_2O) was observed in COPD patients compared to healthy subjects (Table I). COPD patients also showed higher concentrations of white blood cells (7.9±1.9 vs 5.1±1.3 x10^9/l), blood eosinophils (0.41±0.26 vs 0.23±0.15 x10^9/l) and CRP (11.55±11.11 vs 0.7±0.9 mg/l) than healthy subjects. We also observed a reduction of BMI (21.0±2.4 vs 24.5±2.6 kg/m^2) between COPD patients and control subjects, although this did not achieve significance.

Plasma concentrations

Higher concentrations of DROMs (267.8±34.12 vs 36.8±6.0 U.CARR), IL-6 (9.18±2.31 vs 5.82±0.89 pg/ml), TNF-α (11.22±3.67 vs 6.06±1.55 pg/ml) and IL-4 (72.43±10.43 vs 38.71±5.33 pg/ml) were found in plasma of COPD patients compared with healthy subjects (Table I).

The plasma levels of IL-6 and TNF-α were higher in non-reversible COPD (10.72±2.72 vs 7.57±1.52 pg/ml and 13.57±4.49 vs 8.66±1.29 pg/ml) whereas the levels of IL-4 were higher in reversible COPD (78.66±10.22 vs 65.72±10.03 pg/ml) when compared to healthy subjects (Fig. 2-4).

Correlations

The relations between the fat-free mass with the inflammatory markers were studied using the Spearman's rank correlation test, finding negative correlations between fat-free mass and TNF-α in plasma ($p<0.001$, $r=-0.83$), TNF-α in supernatant of induced sputum ($p<0.001$, $r=-0.75$), TNF-α in breath condensate ($p<0.05$, $r=-0.44$) and between PImax, PEmax and TNF-α in plasma ($p<0.01$, $r=-0.61$; $p<0.01$, $r=-0.64$), TNF-α in supernatant of induced sputum ($p<0.05$, $r=-0.50$; $p<0.05$, $r=-0.48$), TNF-α in breath condensate ($p<0.05$, $r=-0.53$; $p<0.05$, $r=-0.46$). Furthermore, a strong correlation was observed between PImax and fat-free mass ($p<0.01$, $r=0.67$).

DISCUSSION

Several recent studies have investigated the inflammation in COPD and the possible mechanisms of its systemic effects (2-5). In agreement with previous studies we found significant increases in several inflammatory biomarkers (CRP, TNF-α, IL-6) (9, 27-37) and leukocytes (9, 27) in the peripheral blood of a group of ex-smoker COPD

patients compared to age-matched healthy subjects. Moreover, we also measured the same inflammatory markers in the supernatant of the induced sputum and in breath condensate in order to compare with the pattern of pulmonary inflammation, and an increase was found in the concentrations of the same inflammatory markers in COPD patients compared to healthy subjects, indicating that in COPD patients pulmonary and systemic inflammation has a similar pattern (32).

Previous studies have characterized the pulmonary inflammation in COPD patients by measuring cells in induced sputum, and have shown an increase in eosinophils in reversible COPD compared to an increase of neutrophils in non-reversible COPD (17). We have confirmed these differences in our own study. We also found higher levels of IL-6 and TNF-α in plasma, in supernatant of the induced sputum and in breath condensate of non-reversible COPD, whereas we found higher levels of IL-4, which is usually associated with allergic inflammation, in patients with reversible COPD. This is the first time that IL-4 has been detected in supernatant of induced sputum and in breath condensate of COPD subjects. We also found higher levels of exhaled NO in COPD patients with reversible airflow limitation, in agreement with a previous study (17).

We also measured pH of the breath condensate and found lower values in COPD patients compared with healthy subjects, in agreement with a previous study (33). We also found a similar reduction in pH values in supernatant of induced sputum confirming acidification of the lower airways in COPD patients. No differences in pH were found between reversible COPD and non-reversible COPD, suggesting that both eosinophilic and neutrophilic inflammation could cause a decrease of pH.

Several studies have highlighted nutritional abnormalities in COPD patients, including alterations in caloric intake, basal metabolic rate, intermediate metabolism and body composition (34-35). Moreover, alterations in body composition may occur in COPD in the absence of clinically significant weight-loss (36). In our study we found a decrease in fat-free mass in COPD patients compared with healthy subjects. We also found a lower fat-free mass in non-reversible COPD than in reversible COPD, although this difference did not achieve statistical significance. We investigated a possible relation between fat-free mass and inflammatory markers and found no correlation with IL-6 and IL-4, but a strong negative correlation between fat-free mass and TNF-α in plasma, sputum supernatant and in breath condensate. Previous reports have highlighted the potential role of TNF-α in inducing catabolic responses in tissues, triggering muscle proteolysis and increased protein degradation (37). Our findings suggest that there could be a relation between inflammation (local and/or systemic) and body composition in COPD patients and this relation, because of its significant correlation with TNF-α, a mediator associated with neutrophilic inflammation, could be more important in non-reversible COPD patients. We found a similar trend in the values of PImax and PEmax: which were lower in non-reversible COPD (although this did not achieve significance) and there was a negative correlation between these measurements of muscle strength with TNF-α concentrations in plasma. This is not surprising as a decrease of fat-free mass can cause a decrease of the muscular strength and so a decrease in the values of the PImax and PEmax (6). Indeed, we confirmed a strong correlation between PImax and fat-free mass. The involvement of TNF-α in the weight loss of COPD has already been investigated, while the other markers (CRP, IL-6) are more associated with cardiovascular complications (37-39).

Finally, we have confirmed a link between inflammation and oxidative stress, which may reflect increased transcription pro-inflammatory cytokine genes in response to reactive oxygen species (39). In COPD there is increased oxidative stress in exhaled breath and in plasma (40). In a previous study we described an increase of oxidative stress and oxidative protein damage in induced sputum of non-reversible COPD compared to reversible (18). In this study we found an increase of the value of plasma DROMs among the COPD patients, particularly among the non-reversible COPD patients, that is in line with our previous data on airways of non-reversible COPD and proved the presence of a concomitant systemic oxidative stress in these patients.

COPD is characterized by an intense inflammatory process in the airways, parenchyma

and pulmonary vasculature, and this process may spill over into the systemic circulation, promoting a generalised inflammatory reaction (31). Our study confirms previous studies of local and systemic inflammation in COPD patients, and distinguishes patterns of inflammation between reversible and non-reversible COPD. The similarity between the pattern and pulmonary inflammation (measured by induced sputum and exhaled breath condensate) and systemic markers of inflammation adds support to the idea that systemic effects arise from a spill-over of inflammation from the lung. Furthermore, these systemic features more closely mimic the neutrophilic pattern of inflammation associated with non-reversible COPD, indicating that this form of COPD is more likely to be associated with systemic effects.

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