Analysis of Oxidative Stress in Exhaled Breath Condensate From Patients With Severe Pulmonary Infections

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OBJECTIVE: Oxidative stress is an intrinsic part of the chain of events leading to inflammation of the airways caused by bacterial infection. The aim of this study was to determine whether analysis of exhaled breath condensate from patients with severe lung infections reveals changes in the redox state at the airway surface.

PATIENTS AND METHODS: The study included a total of 48 subjects divided into 4 groups: individuals without respiratory disease (n=14), patients with multilobar pneumonia (n=13), patients who had chronic obstructive pulmonary disease with superinfection (n=14), and mechanically ventilated patients with severe pneumonia (n=7). A sample of exhaled breath condensate was obtained within the first 72 hours of hospital admission and the concentrations of nitrite, nitrate, 8-isoprostane, and myeloperoxidase (MPO) were determined.

RESULTS: Significant differences in the concentrations of nitrite, 8-isoprostane, and MPO were observed between patients and individuals without respiratory disease but no differences were found between the 3 patient groups. The concentration of MPO was correlated with the concentrations of 8-isoprostane and nitrate, which were normalized to the nitrite concentration.

CONCLUSIONS: Analysis of the concentrations of 8-isoprostane and MPO in exhaled breath condensate allows assessment of oxidative stress in the airways of patients with severe lung infections.

Key words: *Pneumonia. Chronic obstructive pulmonary disease. Reactive nitrogen species. Reactive oxygen species. Pulmonary inflammation. 8-isoprostane. Myeloperoxidase. Nitrate. Nitrite.*

Estrés oxidativo en el condensado exhalado de pacientes con infección pulmonar grave

OBJETIVO: El estrés oxidativo forma parte esencial de la cadena de acontecimientos que conducen al estado inflamatorio de la vía aérea tras la agresión bacteriana. El objetivo del presente trabajo ha sido investigar si el análisis del condensado del vapor exhalado (CER) de pacientes con infección pulmonar grave refleja las alteraciones del estado oxidativo de la interfase aérea.

PACIENTES Y MÉTODOS: Se ha estudiado a un total de 48 pacientes divididos en 4 grupos: sujetos sin enfermedad respiratoria (n = 14), pacientes con neumonía multilobular (n = 13), con enfermedad pulmonar obstructiva crónica sobreinfectados (n = 14) y con neumonía grave ventilados mecánicamente (n = 7). Se obtuvo una muestra de CER en las primeras 72 h tras el ingreso y se determinó la concentración de nitrito, nitrato, 8-isoprostano y mieloperoxidasa (MPO).

RESULTADOS: Se apreciaron variaciones significativas de la concentración de nitrito, 8-isoprostano y MPO en los pacientes respecto del grupo control, pero no entre los diferentes grupos de pacientes. La concentración de MPO se relacionó con las concentraciones de 8-isoprostano y nitrato normalizadas para el valor de nitrito.

CONCLUSIONES: El análisis de la concentración de 8-isoprostano y MPO en el CER permite apreciar el estrés oxidativo en la interfase aérea de los pacientes con infección pulmonar grave.

Palabras clave: *Neumonía. Enfermedad pulmonar obstructiva crónica. Especies reactivas del nitrógeno. Especies reactivas del oxígeno. Inflamación pulmonar. 8-isoprostano. Mieloperoxidasa. Nitrato. Nitrito.*

Introduction

In the normal lung, the equilibrium between antioxidants and oxidants is sufficient to maintain the fluids that cover the surface of the airways and fill the extracellular spaces in a highly reduced state. An increase in the concentration of oxidants or a reduction or excessive consumption of antioxidants leads to the loss of this equilibrium in a situation referred to as oxidative stress. In response to stimuli associated with bacterial infection, especially the production of lipopolysaccharides, macrophages and endothelial cells are activated and express adhesion molecules on their surface, facilitating transmigration of neutrophils from the blood vessels into the alveoli or the airways in general.[1] Activated neutrophils produce a large number of oxidants that usually fall into 2 categories: reactive oxygen species (ROS) and reactive nitrogen species

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(RNS).[2] Production of nitric oxide (NO) by NO synthase generates a nitric oxide radical (NO·) and its derivatives nitrite (NO_2) and nitrate (NO_3). When NO reacts with oxygen it generates the powerful oxidant peroxynitrite (OONO·). Oxidative stress leads to lipid peroxidation in the cell membrane and the formation of a new group of prostanoids called isoprostanes, which are derived from oxidation of arachidonic acid and prostaglandins.[3]

Granulocyte peroxidases such as neutrophil myeloperoxidase (MPO) play an important role in triggering oxidative stress. In neutrophils, hydrogen peroxide generated from the slightly more reactive O_2^- is metabolized by MPO in the presence of chloride ions to form hypochlorous acid, a strong oxidant. While this represents an important antibacterial process, it also has cytotoxic effects.[4,5]

Collection of exhaled breath condensate (EBC) allows samples of the fluid covering the surface of the airways to be obtained by freezing the breath exhaled by the subject. Thus, release of respiratory droplets at the airway surface allows substances dissolved in that fluid to be analyzed in EBC samples.[6] Although the dilution of respiratory droplets in exhaled water vapor does not allow quantitative determination of the biochemical composition of the fluid at the airway surface, it does allow qualitative characterization, especially through the use of internal relationships between biochemical parameters, since despite being difficult to interpret, those relationships are relatively immune to the process of dilution.[7] The analysis of parameters of oxidative stress in EBC has been used with varying degrees of success for the diagnosis of inflammation in chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD),[8,9] asthma,[10,11] interstitial fibrosis,[12] and cystic fibrosis.[13] However, its use has been limited in severe acute bronchopulmonary infections, such as multifocal pneumonia or acute superinfection in COPD.[14]

This study is based on the hypothesis that an inflammatory process of the magnitude of that caused by pulmonary infection should lead to changes in the redox state at the airway surface that can be characterized by an increase in the concentration of oxidants in EBC samples. The aim of the study was to determine whether analysis of EBC allows that hypothesis to be addressed in patients with severe pneumonia and COPD with superinfection. Subjects without respiratory disease were used as controls.

Patients and Methods

Subjects

The study included a total of 48 patients divided into 4 groups:

1. A control group made up of 14 patients admitted for scheduled surgery who had no prior history of respiratory disease, had a normal chest radiograph, and who were either nonsmokers or had given up smoking at least 2 years earlier.

2. A group of patients with multilobar pneumonia, comprising 13 subjects admitted to hospital for pneumonia of varying etiology (7 cases of pneumococcal pneumonia, 1 case of pneumonia caused by *Legionella* organisms, and 5 cases of pneumonia of unknown cause). All of the patients presented with hypoxemia at admission and 2 had a prior history of COPD.

3. A group of 14 COPD patients with superinfection. In 3 cases, the patients also presented bronchiectasis and the infectious agent was found to be *Pseudomonas aeruginosa*; in the remainder, no conclusive microbiological diagnosis was obtained. The criteria proposed by Anthonisen et al[15] were used to establish a diagnosis of bronchial superinfection.

4. A group of 7 mechanically ventilated patients with severe pneumonia were studied in the intensive care unit. Except in 1 patient who was positive for the human immunodeficiency virus and had pneumonia due to *Pneumocystis carinii*, the infectious agent was pneumococcus in all cases.

According to the criteria authorized by the ethics committee of Bellvitge Hospital, informed consent was given by all patients (or family members) after they received information on the study protocol and the aims. Except in the control group, all patients received additional oxygen, with a fraction of inspired oxygen (FiO_2) that ranged from 0.8 in the most severe cases (pneumococcal sepsis, pneumonia caused by *P carinii*, etc) to 0.24 or 3 L/min provided using nasal prongs in the least severe cases. Oxygen therapy was continued in all patients during collection of the breath condensate (in the case of nasal prongs, a change was made to provision of 24% oxygen using a Venturi mask).

Isolation and Processing of EBC

EBC was obtained during the first 72 hours of admission in patients with COPD and pneumonia, immediately following admission in the control group, and within the first 72 hours of diagnosis in the intensive care unit in mechanically ventilated patients with pneumonia. It was obtained at the patient's bedside via freezing of the exhaled vapor using an ANACON condenser (Biostec, Valencia, Spain), which uses a thermoelectric pump to generate low temperatures. Spontaneously breathing patients were connected to the condenser via a unidirectional valve connected to a 45 cm corrugated tube. In mechanically ventilated patients the condenser was inserted in the expiratory circuit 60 cm from the T tube and the humidifying filter was removed (the position of the condenser in the ventilatory circuit is shown in Figure 1). The sample was obtained at temperatures below –10°C over a period of at least 15 minutes. Patients rinsed their mouths prior to each measurement and they were allowed to interrupt the process in order to swallow saliva or when otherwise requested. Provision of oxygen was continued along with the mechanical ventilation conditions where applicable. The sample was defrosted and divided into 0.2 mL aliquots for subsequent biochemical analysis. The aliquots were stored at –80°C prior to analysis.

Analysis of Biochemical Parameters

Total NO_2 and NO_3 were analyzed using a colorimetric assay based on the Griess reaction (Cayman, Ann Arbor, Michigan, USA). The amount of NO_2 was determined by direct addition of Griess reagent (1% sulfanilamide, 0.1%

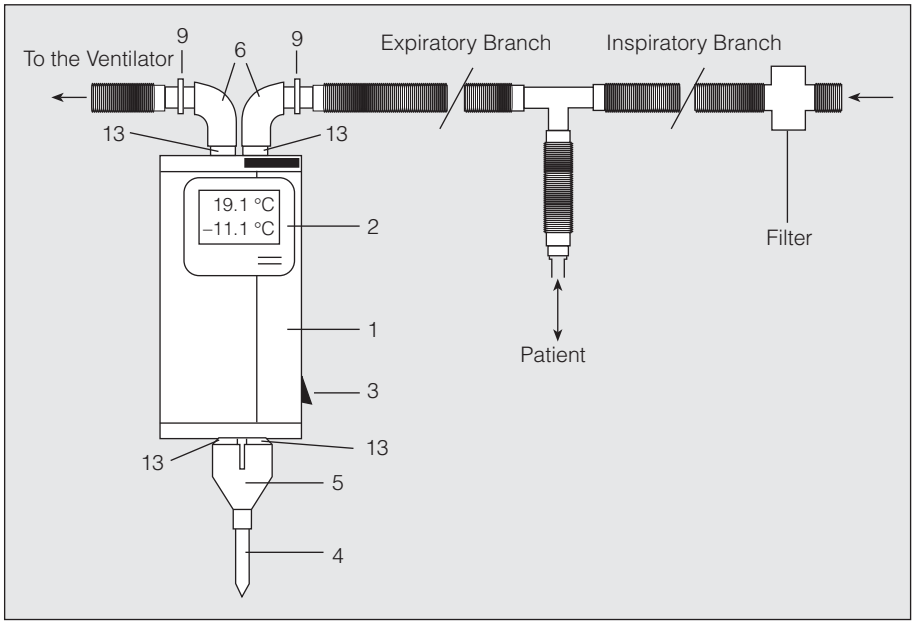

Figure 1. ANACON (Biostec, Valencia, Spain) condenser integrated in the mechanical ventilation circuit. The condenser is inserted in the expiratory branch of the ventilatory circuit via 2 adaptors (*9*) and 2 elastomeric connectors (*6*). The exhaled air passes towards the condensation tubes (*13*) that pass through the body of the condenser (*1*). A Y piece (*5*) closes the circuit with the collection tube for the exhaled breath condensate (*4*). A thermometer (*2*) allows the condensation temperature to be monitored. The apparatus also contains a cooling switch (*3*).

naphthyl ethylene diamine, 2.5% H_3PO_4) to 80 µL of unprocessed sample. The sample was then incubated for 10 minutes at room temperature in the dark and the absorbance was measured at 550 nm. The absolute detection limit of the technique under those conditions was 0.1 µmol/L. Two standard curves with 6 points between 0.1 and 10 µmol/L were constructed in order to obtain the concentration of NO_2 by interpolation. Measurement results below 0.1 µmol/L (undetectable) were assigned a value of 0 (or 0.05 µmol/L for logarithmic calculations).

NO_3 was measured in the same way as NO_2 following enzymatic conversion by addition of NO_3 reductase in the presence of the cofactor reduced nicotinamide adenine dinucleotide phosphate to 80 µL of untreated sample. The sample was first incubated for 1 hour at room temperature and then Griess reagent was added; the remainder of the procedure was as for NO_2. The result gave a total concentration of NO_2 plus NO_3 and from that value the previously obtained concentration of NO_2 was subtracted to calculate the concentration of NO_3.

The concentration of 8-isoprostane was measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit for 8-isoprostaglandin $F_{2\alpha}$ (Cayman). No preconditioning or purification of the samples was performed prior to the assay. Samples (50 µL) were placed in the wells of a microtiter plate, and 50 µL of cholinesterase (8-isoprostane tracer) and 50 µL of anti-8-isoprostane antiserum were added. Following incubation for 18 hours at room temperature in the dark, the wells were washed with buffer and 200 µL of Ellman reagent was added. The plate was then incubated for a further 90 minutes and the absorbance was measured at 420 nm. Two standard curves were prepared for a range of 2 to 32 pg/mL. The sensitivity of the technique under these conditions was sufficient to discriminate 2 pg/mL. Between 2 and 1 pg/mL, the concentration was obtained by reverse extrapolation. In 2 patients from the group with pneumonia, the concentration of 8-isoprostane was not analyzed.

The concentration of MPO in EBC samples was obtained by ELISA using a commercial kit (IBL, Hamburg, Germany). The sensitivity of the technique was 1 U/mL. Two standard curves with 6 points were prepared with a range of 1 to 100 U/mL. Between 1 and 0.1 U/mL, the concentration was obtained by reverse extrapolation. Concentrations below 0.1 U/mL were assigned a value of 0 (or 0.1 U/mL for logarithmic analysis). In 5 patients (3 from the group with multilobar pneumonia and 2 mechanically ventilated patients with pneumonia), the concentration of MPO was not analyzed.

In all cases, samples were analyzed in duplicate and the mean value calculated.

Statistical Analysis

Since the majority of variables did not obey a normal distribution, nonparametric tests, such as the Kruskal-Wallis test for comparison of independent groups, were preferred to parametric analysis of variance. In the figures, the groups are described using their median values and 10th, 25th, 75th, and 90th percentiles (see Figure 2 for description). In the absence of

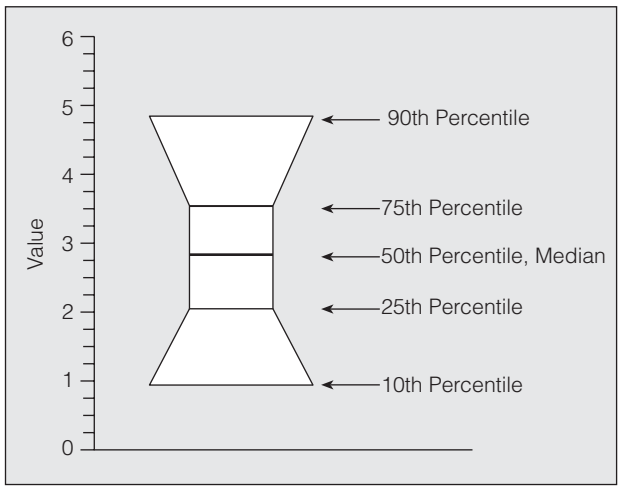

Figure 2. Interpretation of the box graph.

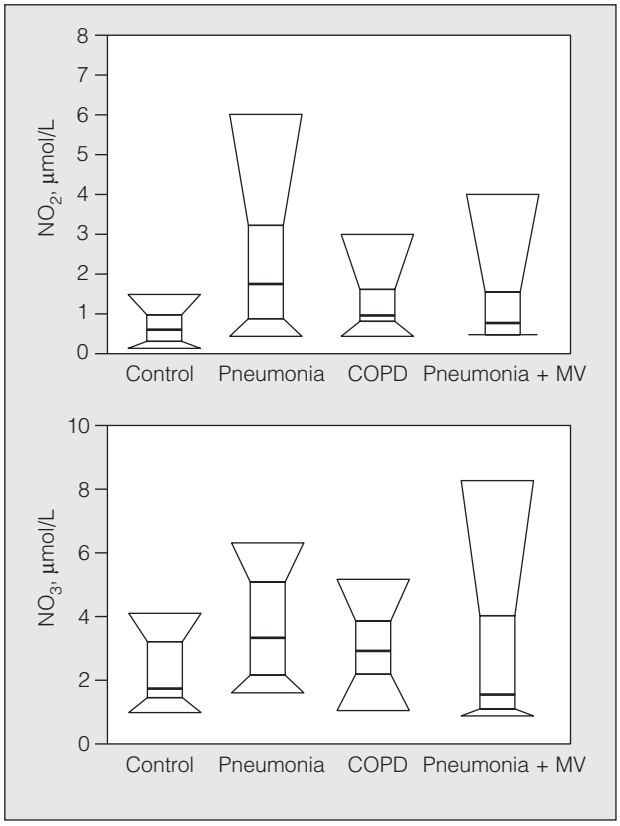

Figure 3. Distribution of the concentrations of nitrite (NO_2) and nitrate (NO_3) in the different study groups. COPD indicates chronic obstructive pulmonary disease; MV, mechanical ventilation.

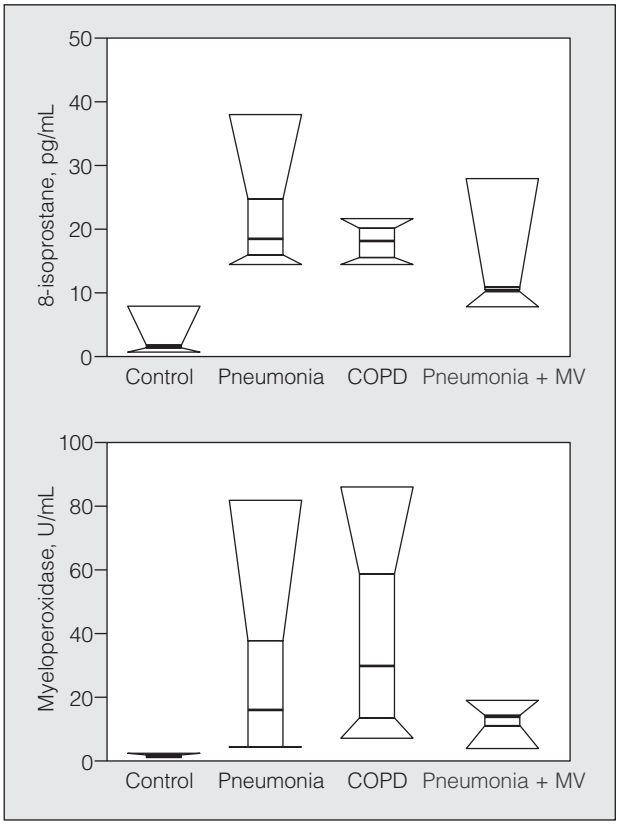

Figure 4. Distribution of the concentrations of 8-isoprostane and myeloperoxidase in exhaled breath condensate from the different study groups. COPD indicates chronic obstructive pulmonary disease; MV, mechanical ventilation.

a normal distribution, no analysis of covariance was performed and the influence of oxygen concentration was assessed using the Spearman rank correlation coefficient. The Pearson correlation coefficient was used in some cases, even though it is a parametric statistic; however, the association was confirmed in all cases using the Spearman correlation coefficient.

Results

The Table displays some of the characteristics of the study population: sex, age, results of blood gas analysis on the day of EBC collection, and body temperature at the time the EBC sample was obtained. The PaO_2/FiO_2 ratio confirmed that oxygenation was significantly disrupted in the 3 groups of patients with respiratory disease.

Figure 3 shows the distribution of NO_2 and NO_3 in the 4 groups. Statistical comparison of the groups revealed significant differences in NO_2 (χ^2=12.2; *P*=.007) but not NO_3 (χ^2=6.63; *P*=.084). The differences in NO_2 between groups were due to the low values observed in individuals without respiratory disease, since analysis of between-group differences for the 3 groups of patients with respiratory diseases did not reveal significant differences (χ^2=3.27; *P*=.19). Figure 4 shows the distribution of MPO and 8-isoprostane concentration in EBC samples from the 4 groups. The analysis revealed significant differences in the concentrations of both 8-isoprostane (χ^2=30.5; *P*<.0001) and MPO (χ^2=30.0; *P*<.0001) due to the increased concentrations observed in samples from patients with respiratory disease compared with the low values (often undetectable) obtained in samples from control subjects. However, no significant differences were observed when the different groups of patients with respiratory disease were compared with

TABLE
Descriptive Statistical Analysis of the Study Groups*

Group	Number	Sex, M/F	Age, Years	PaO_2, mm Hg	$PaCO_2$, mm Hg	PaO_2/FiO_2, mm Hg	ST, °C
Control	14	12/2	62.86 (2.96)	–	–	–	35.9 (0.28)
COPD	14	14/0	68.14 (1.88)	54.92 (8.3)	56.7 (18.3)	226 (47)	36.5 (0.52)
Pneumonia	13	9/4	62.85 (2.96)	56.92 (7.3)	38.7 (4.6)	210 (33)	37.1 (0.9)
Pneumonia with MV	7	7/0	39.86 (14.8)	118.8 (23.0)	41.8 (8.1)	186 (48)	37.9 (37.9)

*Data are shown as means (SD).
M indicates male; F, female; FiO_2, fraction of inspired oxygen; ST, patient temperature during sampling; MV, mechanical ventilation; COPD, chronic obstructive pulmonary disease.

each other (χ^2=5.05, *P*=.079 for 8-isoprostane and χ^2=4.94, *P*=.084 for MPO). No significant relationship was observed between FiO_2 and any of the biochemical parameters studied in EBC samples.

Linear relationships were observed between MPO concentration and the concentration of 8-isoprostane relative to NO_2 (Spearman's *r*=0.417, *P*=.027; Pearson's *r*=0.538, *P*=.001) and between MPO concentration and the NO_3/NO_2 ratio (Spearman's *r*=0.589, *P*=.001; Pearson's *r*=0.4792, *P*=.009) in patients with pulmonary infections (Figure 5).

Discussion

Noninvasive diagnosis of airway inflammation is a complex goal that has been approached using various techniques, with highly variable results in terms of diagnostic efficacy and reliability.[16] No consensus has been reached on the use of EBC, due to the variability of the results obtained and the lack of systematic studies addressing methodological factors that affect its use.[17] Nevertheless, the ease with which it can be used, the absence of associated iatrogenic effects, and the good patient tolerance of the technique all suggest that the clinical conditions appropriate for the use of EBC should be analyzed.

The aim of this study was no other than to determine whether the results of EBC analysis reflect the oxidative stress associated with pulmonary inflammation in cases of severe pulmonary infection. Patients were selected according to the basic criterion of clinically and radiographically diagnosed pulmonary infection, while respiratory failure was used as an indicator of severity. No other selection criteria were used, due to the nonspecific nature of oxidative stress.

The variability inherent in the results of analyzing biochemical parameters in EBC is due to factors associated with the method (nasal and oral contamination, ventilatory pattern, etc), the clinical conditions under which samples are collected (oxygen therapy, mechanical ventilation, etc), or other factors affecting the composition of EBC (dilution, low analyte concentration, etc).[18] Although it has not been possible to standardize some aspects of the methods, which are restricted by the clinical condition of the patients, we can nevertheless discuss the possible influence of some of them.

Nasal and oral contamination are major sources of RNS and, finally, ROS, even when nose clips are used.[19,20] Although the differences between the groups of patients with respiratory disease were not significant, the distribution of NO_3 in the mechanically ventilated patients appeared more similar to that of the control group than to the distribution for the group of nonventilated patients with pneumonia, a finding that may be explained by contamination arising from the upper airways, which produce large amounts of RNS. Apart from this consideration, the pattern observed in patients with tracheal intubation was similar to that of patients with oral respiration, allowing us to conclude that the variations observed had their origin in the lower airways.

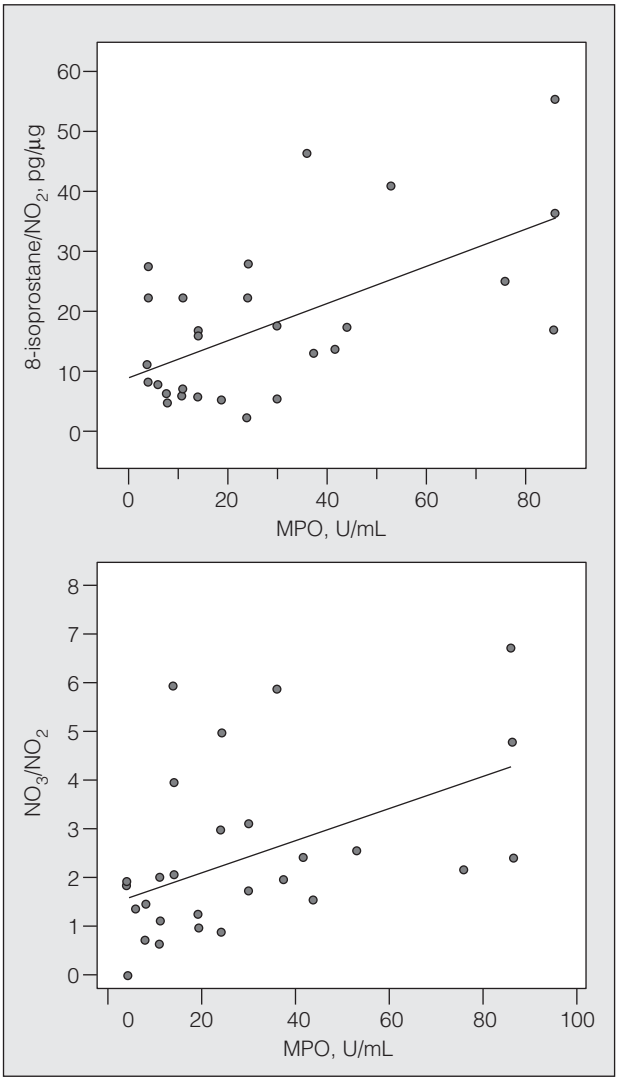

Figure 5. Linear correlations between myeloperoxidase (MPO) and the concentrations of 8-isoprostane and nitrate (NO_3) normalized for the concentration of nitrite (NO_2) in patients with severe pulmonary infection.

Ventilatory pattern influences the amount of EBC collected,[21] but it can also affect its quality, as indicated by the relationship between expiratory flow and the concentration of hydrogen peroxide in EBC[22] and between tidal volume and the concentration of NO_2.[23] Since neither tidal volume nor expiratory flow were measured in free-breathing subjects, adjustments could not be made for this factor. The mechanically ventilated patients, in whom those measurements were available, were too few to evaluate the effect. Consequently, it cannot be ruled out that at least part of the variability observed was due to differences in expiratory flow and the release of respiratory droplets from the airway surface.

FiO_2 is another major confounding factor, since provision of supplementary oxygen leads to an increase in the concentration of factors associated with oxidative stress in EBC from healthy individuals and patients with COPD.[24] The absence of a correlation between

FiO_2 and the concentrations of ROS and RNS does not guarantee that there is no confounding effect. The FiO_2 at the time the sample is obtained is not sufficient to assess the effect of supplementary oxygen on the redox state in the airway, since the effect of oxygen provision is likely to be cumulative.[24] Nevertheless, although increased FiO_2 leads to the presence of ROS in the condensate, its role is, in practice, inseparable from the disease itself in a given clinical setting, and as such, is covered by the main aim of this study.

The high variability of the results obtained in the patients with respiratory disease may also be accounted for by the different stages in the evolution of each of the different diseases studied. Although the composition of EBC was analyzed within 72 hours of admission, the period elapsed since the onset of symptoms is, in fact, highly variable, with prodromes that range from 12 hours to a week in pneumonias and even longer in COPD. The onset of the acute process, although slightly more precise in pneumonias, is difficult to determine in most COPD exacerbations. An analysis in which the point of onset of the symptoms was taken as a reference did not reveal an appreciable tendency in the evolution of any of the parameters in patients with pneumonia. The low number of patients and the imprecision of the variable "days after onset of symptoms" do not allow any interpretation of this observation to be made. Furthermore, no studies have been performed that may serve to guide us in this area.

The concentrations of NO_2 and NO_3 in our control population were similar to those reported for healthy individuals in other studies.[25,26] The same was found for the concentration of 8-isoprostane in control subjects.[27] To our knowledge, no previous studies have analyzed MPO concentration in EBC from healthy individuals; however, the low concentrations of MPO found in induced sputum from healthy subjects (0.1-1 μg/mL)[28] suggests that the concentration in EBC would be practically undetectable in the absence of inflammatory pulmonary disease.

NO is a free radical that plays an important role in the pathophysiology of pulmonary inflammation. The observation that systemic production of NO is increased in the presence of bacterial infection[29] suggests that NO is a marker of local inflammation in pulmonary infections. In an aqueous environment, such as the microenvironment at the airway surface, the generation of NO can be estimated from the concentration of its metabolites NO_2 and its oxidized derivative NO_3. The difference between the groups in terms of the concentration of NO_2 and the sum of NO_2 and NO_3 is indicative of the production of RNS. However, there is no satisfactory explanation for the absence of differences between groups in terms of NO_3 concentration.

The changes observed in condensate from patients with severe pulmonary infection appear to have a greater effect on the production of ROS (8-isoprostane and MPO) than RNS. In animal models, administration of lipopolysaccharides by inhalation leads to a rapid (hours) influx of leukocytes, mainly neutrophils, from the blood.[1] The large number of neutrophils present in the air spaces of patients with pulmonary infections suggests that they may be the principal source of oxidants, but does not rule out the possible existence of other, even exogenous sources, such as the administration of high concentrations of oxygen mentioned earlier.

Shifting the redox balance towards a more oxidizing environment favors peroxidation of membrane lipids. The result is the production of isoprostanes. Unlike most substances of its type, 8-isoprostaglandin $F_{2\alpha}$ is a stable compound, meaning that its concentration can be fairly reliably determined in biological fluids. Isoprostanes have been used as markers of oxidative stress in the lung[30] and their concentration is accepted as an index of lipid peroxidation in vivo.[31] The appearance of isoprostanes in EBC has been linked to ROS-mediated cell toxicity and their concentration has been found to be transiently increased in EBC from patients with exacerbation of COPD[8] and acute lung injury.[32] Unlike isoprostanes, the concentration of MPO has not been studied previously in EBC samples. The increased concentration of MPO observed in this study in EBC samples from all patients with pulmonary infection is suggestive of the importance of both the neutrophil reaction and oxidative activity.

Correlations between EBC parameters could be distorted by dilution effects. Dividing NO_3 and 8-isoprostane concentrations by the concentration of NO_2 allows dilution effects to be eliminated, since they are common to both factors, thereby allowing the index to be interpreted in terms of the ROS/RNS ratio. However, the use of ratios instead of absolute concentrations has the disadvantage that variations can be due to either the numerator or the denominator, and they should be interpreted with caution.[7] For this reason, such ratios have not commonly been used in the analysis of EBC samples. MPO is a product that is almost exclusively related to the production of ROS, while 8-isoprostane is produced through lipid peroxidation caused both by ROS (oxygen, hydrogen peroxide, OH^-, etc) and by RNS (mainly peroxynitrite). NO_3 is generated by oxidation of NO_2 (indicator of the local production of NO). The 8-isoprostane/NO_2 and NO_3/NO_2 ratios can therefore be interpreted as essentially dependent upon an excess of oxidative stress. Consequently, the correlation between MPO and the 8-isoprostane/NO_2 or NO_3/NO_2 ratios reflects the fact that oxidative activity at the airway surface in patients with pulmonary infection is highly correlated with neutrophil activity, as reported previously.[2]

The concentrations of 8-isoprostane and MPO in subjects without respiratory disease are much lower than in patients with respiratory disease and are often practically undetectable. A log-log plot of 8-isoprostane and MPO concentrations in the complete study group reveals 2 clear groups (Figure 6): the first group includes individuals without respiratory disease, while the second group contains the patients from the other 3 groups, with cutoff points of 8 to 10 pg/mL for 8-isoprostane and 2 to 5 U/mL for MPO. Despite the inaccuracies associated with the method (variability, qualitative nature of the

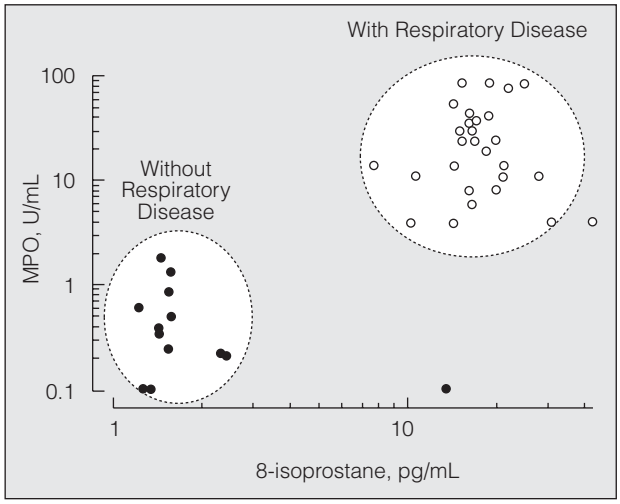

Figure 6. Graph showing the concentration of 8-isoprostane and myeloperoxidase (MPO) in exhaled breath condensate from patients with (circles) or without (dots) respiratory disease. A separation into 2 groups is clearly visible (dashed lines).

measurements, etc), a simultaneous increase in 8-isoprostane and MPO appears to be indicative of an oxidative environment at the airway surface. In a previous study, Carpenter et al[32] found 8-isoprostane concentration to be elevated in patients with adult respiratory distress syndrome, acute lung injury, or sepsis, with a cutoff of 25 pg/mL compared with controls. That value is higher than the one found in this study, a difference that may be accounted for by the technique used. It is noteworthy that the values obtained by Carpenter et al in the reference population were also higher than those reported by van Hoydonck et al[27] in EBC from healthy subjects. Given the high degree of variability inherent in the technique, it appears to be necessary to use more than one factor to define the oxidative state at the airway surface.

In conclusion, analysis of the concentration of 8-isoprostane and MPO in EBC samples allows detection of oxidative stress at the airway surface in patients with severe lung infections.

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