



Original Article

Inflammation Markers in the Exhaled Air of Patients with Bronchiectasis Unassociated with Cystic Fibrosis

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ABSTRACT

Introduction: The aim of the study was to analyse the relationship between the intensity of the respiratory tract inflammation, expressed by oxidative stress markers, and the severity of the disease in patients with bronchiectasis unassociated with cystic fibrosis.

Patients and methods: The study included 25 patients with stable bronchiectasis (15 females and 10 males). As determining factors of severity, the following parameters were collected: degree of dyspnoea, number of exacerbations/admissions in the last year, mean daily sputum volume, sputum colour (graduated colour scale), bacterial colonisation, respiratory function tests, quality of life (St. George questionnaire) and radiological extension of the lesions (Bhalla scale). Inflammation was analysed using the measurement of nitric oxide, pH and concentration of nitrites, nitrates and isoprostane in the exhaled air condensate. The C reactive protein and erythrocyte sedimentation rate were also determined in peripheral blood.

Results: There were no significant relationships between the markers in the exhaled air condensate and the clinical, radiological and functional involvement or the quality of life of the patients. Only bacterial colonisation (16 cases) was associated with higher values of nitrates in exhaled air (mean \pm standard deviation: 18 ± 4 compared to $7 \pm 2 \mu\text{M}$; $r^2 = 0.6$) and a higher number of exacerbations (3.1 ± 1.9 compared to 1.7 ± 1.9 ; $r^2 = 0.3$).

Conclusions: In our study, the measurement of inflammation markers in exhaled air is only associated with some parameters of severity in patients with bacterial bronchiectasis.

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Estudio de marcadores de inflamación en el aire exhalado de pacientes con bronquiectasias no asociadas a fibrosis quística

RESUMEN

Introducción: El propósito del estudio ha sido analizar la relación entre la intensidad de la inflamación de las vías aéreas respiratorias, expresada por marcadores de estrés oxidativo, y la gravedad de la enfermedad en pacientes con bronquiectasias no asociadas a fibrosis quística.

Pacientes y métodos: Se ha estudiado a 25 pacientes con bronquiectasias estables (15 mujeres y 10 varones). Como determinantes de gravedad se recogieron las siguientes variables: grado de disnea, número de exacerbaciones/ingresos en el último año, volumen diario medio de esputo, color del esputo (escala gradual de color), colonización bacteriana, exploración de la función respiratoria, calidad de vida (cuestionario St. George) y extensión radiológica de las lesiones (escala de Bhalla). La inflamación se analizó mediante la medición del óxido nítrico, pH y concentración de nitritos, nitratos e isoprostano en el condensado de aire exhalado. Asimismo se determinaron los valores de proteína C reactiva y la velocidad de sedimentación globular en sangre periférica.

Palabras clave:

Bronquiectasias

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Resultados: No hubo relaciones significativas entre los marcadores en condensado de aire exhalado y la afectación clínica, radiológica, funcional o de calidad de vida de los pacientes. Sólo la colonización bacteriana (16 casos) se relacionó con valores más altos de nitratos en aire exhalado (media \pm desviación estándar: 18 ± 4 frente a $7 \pm 2 \mu\text{M}$; $r^2=0,6$) y un mayor número de exacerbaciones ($3,1 \pm 1,9$ frente a $1,7 \pm 1,9$; $r^2 = 0,3$).

Conclusiones: En nuestra muestra, el estudio de marcadores de inflamación en aire exhalado sólo se relaciona con algunos parámetros de gravedad en pacientes con bronquiectasias colonizadas.

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Introduction

Bronchiectasis, a term used to describe structural alterations with irreversible dilation of the airways, is the end result of multiple diseases which share a common pathological process characterized by inflammation and remodelling of the bronchial wall.¹

The causes leading to the development of bronchiectasis are very diverse and cover a wide spectrum that includes, fundamentally, infectious diseases, above all during the period of lung development, genetic anomalies in the ciliary structures and regulation of mucous secretion, reduction in natural or specific defense mechanisms and autoimmune aggression of the mucosa.^{2,3} The severity and intensity with which the symptoms associated with bronchiectasis manifest (cough, volume of expectorated mucous or dyspnoea) are very variable. Their clinical expression not only depends on the period of lung development in which they are produced, nor only on the extension of the affected airways, but also on the inflammatory or infectious response that the host is able to generate.^{4,5}

Traditionally it has been thought that the common pathogenic mechanism begins by diverse aggressions, which compromise the mucociliary system and transport of mucous.⁶ This facilitates the contact of bacteria with bronchial epithelia and the appearance of a local inflammatory response. This inflammatory response, basically neutrophilic, can sometimes be inadequate or exaggerated and unleash irreversible bronchial damage. The balance between the bacterial load and the inflammatory response, whether local or systemic, is key to understanding the evolution of this process.⁷

Several studies have demonstrated an increase in proinflammatory molecules and cytokines in the serum concentrations and respiratory secretions of bronchiectasis patients, compared to healthy people.⁸⁻¹⁰ However, these studies have not always shown a significant correlation between these mediators and clinical or functional evolution. The majority of published studies have been conducted on patients with cystic fibrosis (CF), a disease in which bronchiectasis is more extensive and the bacterial load, very high.

The relation of inflammatory markers to the clinical course and extension of the disease varies greatly according to the evaluation criteria of the disease and to the markers analyzed. Some studies have found a positive relation to radiological extension,¹¹ while the majority relate them to clinical or functional variables.^{12,13} The discrepancy in the results is likely to be due to the difficulty in defining the severity of the disease whose physiopathological mechanisms are still little known. Multiple clinical, radiological, functional and evolutive parameters have been proposed for severity assessment.^{14,15} The correlation between functional involvement and high resolution computerized tomography findings¹² is high, and therefore the majority of studies regard these as essential elements in severity assessment; nevertheless, other factors such as quality of life, the presence of colonization or number of exacerbations also clearly influence the prognosis of the disease.

In this respect, and in light of the difficulty in finding a specific characteristic to define the severity of bronchiectasis, the majority of studies analyze these variable criteria taking into account the limitations that it entails.

Our objective in this study is to analyze the relation between the intensity of the inflammation of the airways, expressed by oxidative stress markers, and the severity of the disease, defined by a set of clinical, functional and radiological variables, in patients with non-CF bronchiectasis.

Patients and Methods

Study Subjects

We studied a group of adult (> 18 years) patients who attended consecutively the Pneumatology Outpatient Services of 2 centres which participated in this study, and who had been previously diagnosed with diffuse bronchiectasis by computerized tomography.

Patients with bronchiectasis secondary to pulmonary surgical processes, emphysema, allergic bronchopulmonary aspergillosis or diffuse interstitial pulmonary diseases were excluded. All patients underwent a sweat test prior to inclusion in order to exclude bronchiectasis secondary to CF. Chloride concentration superior to 70 (mEq/l.) was considered positive for CF and lower than 45, negative. Intermediate values were discarded by genetic testing.

For inclusion in the study, patients had to be clinically stable for at least the previous 2 weeks, that is, without change in medication or symptoms, emergency room visits or hospitalizations, and must not have received antibiotics during this time period.

Study Design

Information related to anthropometric characteristics (sex, age, weight and height), tobacco habits, comorbidities and types of prescribed drugs was collected from all patients.

Disease severity in patients with bronchiectasis was evaluated using the following parameters: a) lung function involvement; b) anatomical extension of the bronchiectasis; c) volume and characteristics of expectoration; d) resource to health services, and e) impact on strength capacity and health related quality of life.

Lung function involvement was estimated from the results of forced spirometry and lung volumes were measured by the helium dilution method practised on the same day of the first visit. The following variables were collected: forced expiratory volume in one second (FEV₁) as percentage of theoretical value, forced vital capacity (FVC) as percentage of theoretical value, FEV₁/FVC and FEV₁ after bronchodilator administration, and total lung capacity as percentage of theoretical value.

The anatomical extension of the bronchiectasis in the computerized tomography was measured on the modified Bhalla¹⁶ scale, which considers the following characteristics for each lung lobe: extension of bronchiectasis, wall thickness, dilation magnitude, presence or not of plugs and presence of attenuation. Extension was graded as 0 (without bronchiectasis), 1 (one or less than one affected segment), 2 (more than one affected segment) and 3 (cystic bronchiectasis throughout the lobe); bronchial dilation magnitude as 0 (without dilation), 1 (< 2 times the diameter of the adjacent artery) and 2 (> 2 times the diameter of the adjacent artery), and the

bronchial wall thickness as 0 (without increase in thickness), 1 (< 0.5 times the thickness of the adjacent artery), 2 (between 0.5 and 1 times the thickness of the adjacent artery) and 3 (> 1 times the thickness of the adjacent artery). The maximum score was 72 points. All scores were obtained by agreement between 2 of the investigators (M.P.C. and A.D.D.).

In order to analyze sputum characteristics, patients were supplied with 3 sterile containers for the collection of all sputum produced on 3 consecutive days; the arithmetic mean of the 3 days was obtained, expressed in millilitres per day. The colour characteristics of the sputum was analyzed on a mucose colour intensity scale (0-15) developed in our laboratory, which ranges from transparent (0), white (1), progressive intensities of yellow (2-7), green (8-10) and brown (10-15). The colour intensity was determined through agreement on a visual comparison by 2 investigators (M.P.C. and A. D.D.).

Once the characteristics of the sputum had been recorded, the sample was sent for microbiological analysis, which consisted of Gram staining and microbiological culture for habitual microorganisms, bacteria and fungi. Sputum was considered colonized if the analyzed samples were valid (< 10 epithelial cells and > 25 leukocytes per field) and presented more than 10^3 colony forming units. Infection in those patients was clinically ruled out by the absence of recent changes in expectoration or clinical symptoms.

Morbidity produced by bronchiectasi, in terms of recourse to health care services, was determined by the number of exacerbations requiring antibiotherapy in the last year and the number of hospital admissions.

Impact on functional capacity was analyzed by quantifying the dyspnoea, according to the modified Medical Research Council scale and through the study of quality of life. To that end, the patient answered the Spanish version of the St. George's Respiratory Questionnaire (self-administered), which consists of 50 questions divided into 3 domains: symptoms scale (8 items), activity scale (16 items) and impact scale (26 items). The total score ranges from 0 to 100 with high scores indicating a worse health related quality of life.

The determination of inflammatory markers in the airways was performed on samples from exhaled breath condensate on the first visit and before the functional exploration.

To obtain the exhaled breath condensate, the recommendations of the European Respiratory Society were followed, regarding the standardization of the method and patient.¹⁷ An Eco-Screen (Jaegger, Hoechberg, Germany) device was used for measurement. The sample obtained was collected in polypropylene tubes. Once the sample was obtained, and in order to measure the pH of the exhaled air, an aliquot part was separated of at least 2ml and subjected to deaeration during 10 minutes with an inert gas free of carbon dioxide (helium), at a flow of 2l/min. Samples whose carbon dioxide values were less than 5mmHg were considered valid. The pH was determined immediately by pH electrode (Radiometer, Copenhagen, Denmark).

The unused sample was frozen at -70° C in aliquot parts of 200 μ l for later determination of the remaining markers. The concentration of nitrites and nitrates in the exhaled breath condensate was obtained by colourimetry using the Griess reaction (Cayman Chemical) and the results were expressed in micromoles. The concentration of 8-isoprostane was obtained by enzymeimmunoanalysis (Cayman Chemical) and expressed in picograms/millilitres.

All patients were also tested to determine the fraction of nitric oxide (NO) in the exhaled air (FE_{NO}) using a chemoluminescence analyzer (model Logan Research 2000), according to Kharitonov's method, and the results expressed in parts per billion (ppb).

Reactive C-protein and the speed of globular sedimentation were determined as acute-phase reactants in blood.

Statistical Analysis

First, a descriptive analysis was made of the sample for all the recognized variables. The values were expressed as mean and standard deviation or as a percentage. The differences between colonized and non-colonized patients was established using the Mann Whitney U test or the χ^2 test according to the type of variable. Both groups were proved to be similar in age, sex and drugs used. In order to analyze the relation between inflammatory markers and disease intensity, a Spearman correlation matrix was used and the correlations analyzed for the whole group and after adjustment for the presence of colonization. Differences with a value of $p < 0.05$ were considered statistically significant. The analyses were performed using SPSS software version 10.6 (SPSS for Windows, Chicago, U.S.A., 1999).

Results

25 patients were included (15 women and 10 men) with an average age of 58 years. Their characteristics for both functional situation and bronchiectasis severity are shown in Tables 1 and 2. 64% were colonized, the most frequently isolated microorganisms being *Pseudomonas aeruginosa* and *Haemophilus influenzae*. 4 patients had colonization associated with *Aspergillus fumigatus*.

The differential analysis between patients with bronchiectasis showed a significantly higher number of exacerbations in the last year in the colonized bronchiectasis group (Table 3). No differences were found in either group as far as the medication used that may have modified the results. With regard to inflammatory parameters, an increase was observed in nitrate concentrations in the exhaled breath condensate in patients with colonized bronchiectasis (mean \pm standard deviation: 18.9 ± 16.4 versus 7.1 ± 5.6 μ M).

On overall analysis of all patients in relation to the inflammatory markers in the airways and disease intensity, we found no statistically significant association in the values dependent on the individual (functional capacity, quality of life, dyspnoea) nor in the extension and severity of the bronchiectasis itself.

After adjusting for the presence of colonization, a significant inverse correlation was observed between FEV_1 values and the presence of high FE_{NO} values, and there was a significant positive

Table 1
Anthropometric and functional characteristics of patients with bronchiectasis

No. of patients	25
Age (years)	58 \pm 12
Sex men/women	10/15
BMI (kg/m ²)	26 \pm 5
Smokers (%)	4 (16%)
FEV_1 (%pred.)	60.7 \pm 23.8
FEV_1 after BD (%pred.)	61.2 \pm 21.2
FEV_1/FVC	0.62 \pm 0.23
TLC (%pred.)	91 \pm 23
Exacerbations/year	2.6 \pm 2.5
Admissions/year:	0.4 \pm 0.6
Dyspnoea	1.7 \pm 0.9
St. George Questionnaire	47 \pm 17
Symptoms	50 \pm 24
Activity	41 \pm 17
Impact	55 \pm 19
N.º of patients in treatment	
Inhaled steroids	9
Long duration B ₂	17
N-acetylcysteine	4

Data are expressed as mean \pm standard deviation or number (percentage) of patients.

BD: bronchodilator; FEV_1 : forced expiratory volume in one second; FVC: vital forced capacity; BMI: body mass index; TLC: total lung capacity.

Table 2

Severity of bronchiectasis and inflammatory markers in exhaled breath condensate and blood

HRCT, Bhalla scale	25.6 ± 15.4
Sputum volume (ml)	24 ± 18
Sputum colour	12 ± 4
Sputum colonization	
Normal flora	9
<i>Haemophilus</i>	4
<i>Pseudomonas</i>	12
<i>Aspergillus</i>	4
GSV (mm/h)	44 ± 36
CRP (mg/dl)	16 ± 18
FE _{NO} (ppb)	21 ± 18
pH in condensate	6.2 ± 0.8
8-isoprostane (pg/ml)	1.3 ± 0.5
Nitrites (μM)	15.7 ± 9.4
Nitrates (μM)	14.7 ± 14.3

Data are expressed as mean ± standard deviation. FE_{NO}: fraction of exhaled nitric oxide; CRP: C-reactive protein; HRCT: high resolution computerized tomography; GSV: globular sedimentation velocity.

Table 3

Differences in disease severity and inflammatory markers between patients with and without colonization

	Colonized	Non colonized	P
N.º of patients	9	16	
Age (years)	55 ± 14	60 ± 10	U
Sex men/women	5/6	5/9	U
Smokers (%)	2 (18%)	2 (32%)	U
BMI (kg/m ²)	24 ± 4	26 ± 5	U
FEV ₁ (%pred.)	71 ± 29	54 ± 18	U
FEV ₁ after BD (%pred.)	72 ± 21	56 ± 14	U
FEV ₁ /FVC	0.64 ± 0.31	0.61 ± 0.16	U
TLC (%pred.)	89 ± 23	92 ± 24	U
HRCT, Bhalla scale	21 ± 16	27 ± 15	U
Exacerbations/year	1.7 ± 1.9	3.1 ± 1.9	0.05
Admissions / year:	0.4 ± 0.7	0.3 ± 0.6	U
Dyspnoea (MRC)	1.5 ± 1.3	1.8 ± 0.7	U
St. George	37 ± 21	51 ± 14	U
Symptoms	38 ± 32	51 ± 19	U
Activity	30 ± 14	46 ± 16	U
Impact	46 ± 14	58 ± 24	U
Sputum volume (ml)	29 ± 26	21 ± 14	U
Sputum colour	11 ± 3	13 ± 3	U
GSV (mm/h)	46 ± 29	43 ± 41	U
CRP (mg/dl)	22 ± 25	22 ± 14	U
FE _{NO} (ppb)	33 ± 23	14 ± 7	U
pH	6.8 ± 0.3	6.8 ± 0.4	U
8-isoprostane (pg/ml)	1.1 ± 0.3	1.4 ± 0.6	U
Nitrites (μM)	15.6 ± 11.1	15.7 ± 8.8	U
Nitrates (μM)	7.1 ± 5.6	18.9 ± 16.4	0.05

Data are expressed as mean ± standard deviation or number (percentage) of patients.

BD: bronchodilator; FE_{NO}: fraction of exhaled nitric oxide; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; BMI: body mass index; MRC: Medical Research Council scale; NS: not significant; CRP: C-reactive protein; HRCT: high resolution computerized tomography; TLC: total lung capacity; GSV: globular sedimentation velocity.

correlation of nitrates with FEV₁, the number of exacerbations and the intensity of dyspnoea (Table 4).

In addition to the parameters studied, we observed a statistically significant correlation between the quality of life parameters and respiratory function involvement, independently of the presence or not of colonization. Consequently, the result of the St. George questionnaire, both in the total score and in that of the 3 sub-scales, is associated with lower respiratory function and a greater number of exacerbations. The colour intensity of sputum is associated with a greater number of exacerbations. Admission and exacerbations are fundamentally associated with poorer respiratory function and quality of life.

Table 4

Correlation coefficients between airway inflammation parameters and bronchiectasis severity criteria in colonized patients

	FE _{NO}	pH	8-isoprostanes	Nitrates
FEV ₁ (%pred.)	-0.64	0.18	0.03	0.59
FEV ₁ after BD (%pred.)	-0.66	0.08	0.22	0.70
HRCT, Bhalla scale	0.10	0.18	0.56	0.05
Admissions / year:	0.28	0.10	0.08	0.17
Exacerbations/year	0.24	0.08	0.20	0.60*
Dyspnoea (MRC)	0.23	0.18	0.27	0.71*
St. George questionnaire	0.08	0.24	0.13	0.06
Symptoms	0.21	0.54	0.27	0.05
Activity	0.09	0.33	0.17	0.08
Impact	0.04	0.23	0.39	0.37
Sputum volume (ml)	0.13	0.14	0.12	0.22
Sputum colour	0.32	0.15	0.11	0.44

BD: Bronchodilator; FEV₁: forced expiratory volume in one second; MRC: Medical Research Council scale; HRCT: high resolution computerized tomography.

*p < 0.05.

Discussion

The results of this study show that, in non-CF bronchiectasis, the relation between the severity of the disease and some oxidative stress markers is conditioned by the presence of colonization of the airways.

This relation is expressed differently in patients with respect to the inflammatory markers used: while the FE_{NO} maintains an inverse relation with the degree of obstruction, nitrate concentration is a final marker of oxidative stress and is not only related with functional involvement, but also with a greater relapse rate.

Mucosal inflammation of the airways in bronchiectasis patients is traditionally characterized by a cellular predominance of neutrophils. The exaggerated presence of neutrophils, and, therefore, the activity of their enzymes, such as myeloperoxidase, is the origin of greater acidification of the airways as a consequence of the release of the superoxide anion. Among the mechanisms of oxidative stress that go into action after the production of superoxide anion is the exaggerated production of hydrogen peroxide and the compounds derived from lipid peroxidation, as in the case of 8-isoprostane. Oxidation of NO-derived products gives rise to an increase in their metabolism and the formation of more stable products like nitrites and nitrates.

The acidification of respiratory secretions constitutes one of the first manifestations of the increase in oxidative stress. In patients with chronic obstructive lung disease (COPD) or bronchiectasis, a decrease in pH in the exhaled breath condensate is observed when compared with healthy individuals or patients with other airway involvements like asthma.¹⁸ In works which have observed this, the decrease in pH in bronchiectasis patients maintains a significant relationship with the presence of hydrogen peroxide and the degree of airway obstruction; nevertheless, a significant correlation between acidification and 8-isoprostane values or the concentration of nitrites/nitrates in exhalation from the airways has not been shown. The decrease in pH values in exhaled breath condensate was significantly greater in patients colonized by *P. aeruginosa*. In our study, no differences in pH were found between patients who presented colonization and those who did not, nor was any relation observed to the disease severity parameters. The exclusion of CF patients and the stability of our patients may contribute to these findings. Moreover, the determination of the pH in exhaled breath condensate is a very variable value in the majority of the studies.^{11,18,19}

NO is a biological mediator formed as a result of the action of the enzyme NO synthetase, which is found in the majority of airway cells, whether constitutive or inflammatory. The utility of its measurement in patients with bronchiectasis is still not well established. The works of the Kharitonov group¹¹ showed that, as

occurs in other chronic inflammatory diseases of the airways, like COPD or asthma, FE_{NO} values were increased in patients with bronchiectasis; other studies,²⁰⁻²³ however, have shown that in patients with bronchiectasis secondary to CF or ciliary dyskinesia, the concentration of NO in exhaled air was not increased even during infectious exacerbations. Indeed, it has been suggested that some normal values of FE_{NO} may serve to differentiate and diagnose the involvements of other chronic inflammatory diseases of the airways. In the study of Ho et al.,²⁰ FE_{NO} concentrations in patients with bronchiectasis were no different from those recorded in healthy individuals.

NO is formed in epithelial and inflammatory cells of the bronchial mucosa and has to diffuse through the mucous secretions to reach the lumen of the airways. The presence of abundant viscous secretions in the airways, as occurs in bronchiectasis, makes intraluminal diffusion difficult while facilitating its reaction with other reactive species to form nitrites/nitrates. According to this hypothesis, the greater the production of mucous, the lower the concentration of NO in exhaled breath condensate and the greater the nitrate concentration. In this work we observe that NO values were inversely correlated to the obstruction of the airways, so that the less obstructed the patient, the greater the quantity of NO. This fact may indirectly uphold the hypothesis suggested earlier that in patients with bronchiectasis the obstruction is related to the presence of abundant secretions; nevertheless, no relation was found to the volume of sputum collected.

The compartmental analysis of FE_{NO}, differentiating the alveolar and bronchial fraction, has also permitted the differences between the causes of bronchiectasis to be demonstrated. Thus, in patients with bronchiectasis associated with primary ciliary dyskinesia, reduction is produced fundamentally at the expense of the bronchial component, as might be expected according to the diffusion difficulty hypothesis, while in patients with another kind of bronchiectasis the bronchial component is similar or superior to that of healthy controls.²³ As far as the alveolar component, there are no differences between the healthy controls and patients with bronchiectasis or ciliary dyskinesia.

The low production of NO reduces its bactericidal activity and facilitates the appearance of exacerbations. In our work, no differences were found between exacerbations and NO concentration, but a relation was observed between the concentration of nitrates and the number of exacerbations in the group of colonized patients.

Nitrite/nitrate concentration in airway secretions in patients with bronchiectasis is related to the greater reaction capacity of NO. The greater the amount of secretion, or during an infection, the greater the production of nitrates observed. In CF-related bronchiectasis, values have been observed to be significantly higher during exacerbation, but not in patients in a stable situation or in the healthy controls.²⁴ In the study of Ho et al.,²⁵ nitrite values in the exhaled breath condensate were also increased in patients with CF when compared to the healthy group.

With regard to the rest of the markers studied, we know that bacterial infections contribute to oxidative stress by enhancing the recruitment and activation of macrophages and facilitating the release of hydrogen peroxide and 8-isoprostane. In patients with inflammatory diseases of the airways, such as COPD,²⁶ an increase in 8-isoprostane values has been observed in exhaled breath condensate, but no relation could be demonstrated to the degree of obstruction or the intensity of neutrophilic inflammation. In patients with chronic bronchiectasis colonized by *P. aeruginosa*, the concentration of hydrogen peroxide was found to be increased and related to the extension and severity of the disease.²⁷ In our work we have found no differences in 8-isoprostane values between colonized and non-colonized patients, nor any relation to the severity parameters of bronchiectasis. In some of the earlier studies²⁸ the relation was not modified by antibiotic or anti-inflammatory treatment which

indicated a greater inflammation that probably does not exist in our sample, which explains the differences in relation to the findings of the said study.

Oxidative stress changes in patients with bronchiectasis have been shown not only in the airways but also in the way inflammatory blood cells react against infection. In a study performed by King et al.²⁹ in 103 adults with idiopathic bronchiectasis, the release of oxygen-reactive products in neutrophils stimulated by *Staphylococcus aureus* was seen to be lower in patients with bronchiectasis. According to those authors, there is an intrinsic reduction similar to that which occurs in chronic granulomatous disease, which would justify the predisposition to infection and the appearance of bronchiectasis.

In the analysis of the patients included in our sample it is also possible that the treatments used have modified the results. In this respect, we found no differences in the percentage of patients who used inhaled corticoids between both groups. Given the low number of patients who took N-acetylcysteine, it was difficult to adjust the results for this variable. The relation that might have occurred in these patients, at a daily dosis of 600mg, has not been analyzed in our results.

The performance of other analyses in terms of variables such as radiological extension or degree of airway obstruction was discarded due to the absence of differences between the groups of patients and the small sample size.

In summary, the changes in inflammatory response that accompany bronchiectasis are very variable in terms of the presence or not of bacterial colonization and oxidative stress response. In our study, no differences could be found in oxidative stress response in patients with colonized or non-colonized bronchiectasis, except for differences produced by a lower diffusion of NO and the capacity to generate nitrates. The usefulness of the inflammatory markers studied was reduced by the absence of a relation with the remaining markers of disease severity, and should be limited to the characteristics of the patients included in our study and should not, therefore, be compared to other groups such as patients with CF or milder bronchiectasis.

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