Exhaled H$_2$O$_2$ in Steady-State Bronchiectasis*

Relationship With Cellular Composition in Induced Sputum, Spirometry, and Extent and Severity of Disease

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**Study objectives:** To determine the concentration of exhaled H$_2$O$_2$ in patients with bronchiectasis, and to study the relationship between levels of exhaled H$_2$O$_2$, extent of disease, symptoms score, spirometry, and cellular composition obtained from induced sputum; furthermore, to account for possible confounding effects of inhaled corticosteroids (ICS) usage, long-term oral antibiotic treatment, and chronic colonization with *Pseudomonas aeruginosa*.

**Design:** Cross-sectional study.

**Patients:** Thirty patients with steady-state bronchiectasis.

**Results:** Mean (95% confidence interval [CI]) exhaled H$_2$O$_2$ levels were significantly elevated in patients with bronchiectasis compared to normal subjects: 1.1 (0.87 to 1.29) µM vs 0.3 (0.19 to 0.36) µM, respectively (p < 0.0001). Patients treated with ICS had similar values as steroid-naïve patients. The group of patients with *P. aeruginosa* colonization showed a significantly increased concentration of H$_2$O$_2$ compared to the group without *P. aeruginosa* colonization. Patients receiving long-term oral antibiotic treatment had significantly higher values of H$_2$O$_2$ compared to those not receiving antibiotics. There was a significant positive correlation between H$_2$O$_2$ and the percentage of neutrophils in induced sputum or the extent of the disease as defined by high-resolution CT. A significant negative correlation was found between H$_2$O$_2$ and FEV$_1$ percent predicted. Finally, there was a significant positive correlation between H$_2$O$_2$ and the symptoms score.

**Conclusions:** Patients with bronchiectasis in stable condition showed increased levels of exhaled H$_2$O$_2$. The above-mentioned levels were not decreased either by ICS or long-term oral antibiotic treatment, but were significantly affected by chronic colonization with *P. aeruginosa*. H$_2$O$_2$ levels could be an indirect index of neutrophilic inflammation, impairment of lung function, and extension and severity of the disease.

*(CHEST 2002; 121:81–87)*

**Key words:** bronchiectasis; hydrogen peroxide; induced sputum; *Pseudomonas aeruginosa*; spirometry

**Abbreviations:** BSS = bronchiectasis severity score; CI = confidence interval; HRCT = high-resolution CT; ICS = inhaled corticosteroids; ROI = reactive oxygen intermediate

Bronchiectasis is a chronic suppurative lung disease of diverse etiology characterized by irreversible dilation of the bronchi and persistent purulent sputum production. High levels of proinflammatory cytokines are present in airway secretions, and neutrophils are the predominate cells in the airway lumen. In patients with bronchiectasis, bronchial damage is thought to exist due to neutrophil inflammatory products released in response to bacterial infection. The patient’s condition can be monitored by lung function tests and high-resolution CT (HRCT), although the latter requires exposure to radiation. Chronic colonization with *Pseudomonas aeruginosa* is associated with extensive lung disease and severe airflow obstruction.

Induced sputum is a relatively noninvasive, reliable, valid, and responsive technique to measure airway inflammation. It can be applied even in the more severe conditions, and can be used repeatedly to investigate the inflammatory pattern as well as the effects of treatment on various diseases.
Oxidative stress, defined as an increased exposure to oxidants or decreased antioxidant capacities, is implicated in airway inflammatory diseases including bronchiectasis.\textsuperscript{7,8} Oxygen-derived free radicals or metabolites, collectively termed reactive oxygen species, are important mediators of cell and tissue injury during inflammation and may be produced by several types of inflammatory cells.\textsuperscript{9} It has been shown\textsuperscript{7} that the increased oxidative burden in the lungs of patients with bronchiectasis is being reflected by increased levels of exhaled H\textsubscript{2}O\textsubscript{2}.

In testing the hypothesis that reactive oxygen intermediates (ROIs) such as H\textsubscript{2}O\textsubscript{2} are important in bronchiectasis,\textsuperscript{7,8} we measured H\textsubscript{2}O\textsubscript{2} concentrations in the expired air of patients with bronchiectasis. To account for possible confounding effects of the use of inhaled corticosteroids (ICS), long-term oral antibiotic treatment, or chronic colonization with \textit{P. aeruginosa}, further analysis was conducted after subclassifying the patients on the basis of treatment with ICS, oral antibiotics, and chronic colonization with \textit{P. aeruginosa}.

### Materials and Methods

#### Subjects

Fifteen normal subjects (12 men; mean [SD] age, 34 [8] years; range, 20 to 46 years; Table 1) were recruited from the staff of our department. All were nonsmokers and were free of both upper and lower respiratory tract infections for at least 6 weeks before this study. They had no history of chronic disease and were not receiving any regular medication. The subjects had a negative history of allergy (negative skin prick test results to common allergens), normal spirometry (FEV\textsubscript{1}, 95 [8]% predicted; range, 86 to 110% predicted), and normal bronchial reactivity with a provocative concentration of histamine causing a 20% fall in FEV\textsubscript{1} > 0.800 mg in all subjects.

Thirty patients (24 men; mean [SD] age, 39 [16] years; range, 19 to 58 years; FEV\textsubscript{1}, 69.5 [20]% predicted; range, 27 to 101% predicted) with diagnosed bronchiectasis were recruited from the outpatient clinic at Athens Army General Hospital (Table 1). All patients had a negative history of allergy (negative skin prick test result to common allergens). None of our patients had reversibility with inhaled salbutamol of ≥12% of predicted FEV\textsubscript{1}. All patients had bronchiectasis diagnosed on the basis of clinical and radiologic features and confirmed by HRCT of the thorax. Patients were included in the study only if they were in clinically stable condition and had no evidence of acute infective exacerbation (lower or upper airways) for at least 4 weeks prior to the study. Ten patients with chronic colonization with \textit{P. aeruginosa} were separately studied and compared with the remaining 20 patients who had no evidence of any chronic colonization with bacteria (Table 1). Chronic colonization with \textit{P. aeruginosa} was classifiable on the basis of treatment with ICS, oral antibiotics, or chronic colonization with \textit{P. aeruginosa}.

#### Table 1—Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal Subjects (n = 15)</th>
<th>All (n = 30)</th>
<th>ICS (+) (n = 14)</th>
<th>ICS (−) (n = 16)</th>
<th>PA Group (n = 10)</th>
<th>PA Group (−) (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female sex, No.</td>
<td>12/3</td>
<td>24/6</td>
<td>10/4</td>
<td>14/2</td>
<td>8/2</td>
<td>16/4</td>
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<tr>
<td>Age, yr</td>
<td>34 (8)</td>
<td>39 (16)</td>
<td>37 (11)</td>
<td>41 (20)</td>
<td>42 (13)</td>
<td>37.5 (18)</td>
</tr>
<tr>
<td>FEV\textsubscript{1} % predicted</td>
<td>95 (8)</td>
<td>69.5 (20)</td>
<td>71 (16)</td>
<td>69 (23)</td>
<td>59 (25)</td>
<td>72 (22)</td>
</tr>
<tr>
<td>FVC % predicted</td>
<td>97 (7)</td>
<td>78 (14)</td>
<td>79 (12)</td>
<td>77 (16)</td>
<td>67 (9)</td>
<td>83.5 (17)</td>
</tr>
<tr>
<td>HRCT score</td>
<td>Not done</td>
<td>9.5 (4.5)</td>
<td>10 (5)</td>
<td>9 (4)</td>
<td>14 (3)</td>
<td>8 (4)</td>
</tr>
<tr>
<td>Range</td>
<td>2–18</td>
<td>2–18</td>
<td>2–17</td>
<td>7–18</td>
<td>2–14</td>
<td>2–14</td>
</tr>
<tr>
<td>Disease duration, yr</td>
<td>11 (8)</td>
<td>13 (9)</td>
<td>9 (7)</td>
<td>14 (9)</td>
<td>9.5 (7.5)</td>
<td>9.5 (7.5)</td>
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<tr>
<td>BSS</td>
<td>5 (1.7)</td>
<td>5 (2)</td>
<td>4.5 (1.3)</td>
<td>6 (1.4)</td>
<td>4 (1.4)</td>
<td>4 (1.4)</td>
</tr>
<tr>
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<td>2–8</td>
<td>2–8</td>
<td>2–7</td>
<td>3–8</td>
<td>2–8</td>
<td>2–8</td>
</tr>
<tr>
<td>IE/previous year</td>
<td>4 (2)</td>
<td>3 (1.5)</td>
<td>5 (3)</td>
<td>5 (3)</td>
<td>3.5 (2)</td>
<td>3.5 (2)</td>
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<tr>
<td>Range</td>
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<td>2–7</td>
<td>2–8</td>
<td>2–8</td>
<td>2–6</td>
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</tr>
</tbody>
</table>

*Data are presented as mean (SD) unless otherwise indicated.

IE/previous year = infective exacerbations in the previous year; ICS (+) = patients treated with ICS; ICS (−) = patients not treated with ICS; PA group = patients with \textit{P. aeruginosa} colonization; PA group (−) = patients without \textit{P. aeruginosa} colonization.
defined as more than three isolations of *P. aeruginosa* from separate samples over 3 months. All patients had negative sweat test results. Patients with cystic fibrosis, allergic bronchopulmonary aspergillosis, asthma, α₁-antitrypsin deficiency, COPD, and atopic diseases were excluded. Asthma and COPD were excluded by using the American Thoracic Society criteria. None of our patients were current smokers. Two patients had stopped smoking >3 years prior to the study (mean smoking history, <4 pack-years), and the remaining 28 patients had a negative smoking history. Twenty-one patients were receiving regular inhaled β₂-agonists (short or long acting) and 14 patients were receiving ICS (fluticasone propionate, 500 to 1,000 μg/d) or budesonide, 400 to 800 μg/d. Eleven patients received long-term oral antibiotics (5 patients in the group with *P. aeruginosa* colonization). No attempt was made to modify their treatment with inhaled steroids and long-term oral antibiotics, which remained unchanged for at least 3 months before sputum induction and H₂O₂ collection. None of the patients were receiving inhaled or oral mucolytics, and none were receiving oxygen therapy. The study protocol was approved by the ethics committee of our hospital, and all subjects gave written informed consent.

**Assessment of Disease Severity**

Symptoms were assessed as previously described using a different analog scale score. Patients were instructed to record accurately their daily symptoms (wheezing, sputum production, cough) for a 2-week period. Symptoms were scored by the patient every day using a subjective 0 to 3 scoring system, where 0 = none, 1 = mild, 2 = moderate, and 3 = severe. Sputum was collected over a 24-h period and was stored in clear plastic pots (50 mL). Sputum production was scored as follows: 0 = no sputum, 1 = less than a half pot, 2 = more than a half pot but less than a full pot, 3 = more than a pot per day. The symptoms were added for each day, and a mean daily score was calculated for the period of assessment (score range, 0 to 9).

**Lung Function**

FEV₁ and FVC were measured using a dry spirometer (Vic-test, model VEP2; Mijnhardt; Rotterdam, Holland). The best value of three maneuvers was expressed as a percentage of the predicted value. Airway responsiveness was measured only in normal subjects by histamine provocation challenge. The provocative concentration of histamine causing a 20% fall in FEV₁ was calculated by linear interpolation of the semilogarithmic dose-response curve.

**HRCT**

A recent HRCT (within a week from entering the study) scan (GE 9500 Highlight Advanced; General Electric Medical Systems; Milwaukee, WI) of 30 patients was assessed and scored by the same radiologist, who was blinded to all other details concerning the patients’ clinical and functional conditions. During the 7-day interval, none of our patients had an exacerbation of disease. Each lobe of both lungs was graded for bronchiectatic changes on a scale of 0 to 3 (lingula was scored as a separate lobe), giving a maximum of 18 points (0 = no bronchiectasis, 1 = one bronchopulmonary segment involved, 2 = more than one bronchopulmonary segment involved, and 3 = gross cystic bronchiectasis). Patients with emphysematous changes were excluded. This scoring has been used in a previous study with low interobserver variation.

**Collection of Expired Breath Condensate and H₂O₂ Measurement**

A heat-exchanger unit (RHES, model 6V3; Jaeger; Wuerzburg, Germany) was used to produce cold air of −15°C to −18°C at an air flow of 80 L/min. A double-jacketed glass tube of 30 cm in length was specifically adapted to the cold-air system and a two-way unidirectional valve (Series 2-200; Hans Rudolph; Kansas City, MO) was connected to the tube in order to separate inspiration from expiration. After rinsing their mouths, the subjects were comfortably seated in a chair wearing nose clips and breathed in a relaxed manner (tidal breathing) for 10 min. The breath condensate was collected at the other end of the tube and was immediately stored at −70°C for later analysis. According to this design, salivary contamination was highly unlikely and was easily observed, as the proximal cold-air connection was 20 cm away from the mouthpiece. Approximately 1 mL of breath condensate was collected in a 2-mL sterile plastic tube. H₂O₂ measurements were performed the same day (minimum of 2 days and maximum of 20 days from collection time in all samples). To examine the repeatability of H₂O₂ measurements within subjects, condensate was collected from 5 normal subjects and 10 patients on 2 consecutive days. To assess the stability of H₂O₂ in the frozen condensate, 4 mL of condensate was collected from eight subjects (four patients). The above-mentioned concentration was divided into 1-mL aliquots, in which H₂O₂ concentrations were determined after 2 days, 1 week, 2 weeks, and 3 weeks of storage (the maximum time between collection and measurement in the whole samples). Repeatability of extracted H₂O₂ measurements and stability of H₂O₂ frozen samples were estimated as previously described.

All condensate samples were tested for salivary contamination by determination of amylase activity. Amylase activity was carried out spectrophotometrically (kinetic method) using a commercial reagent kit (model 981362; KONE Instruments; Espoo, Finland). In this procedure, α-amylase of the sample and the enzyme α-glucosidase hydrolyzes the substrate p-nitrophenyl-α-D-malto- heptaoside to glucose and p-nitrophenol. The liberation of p-nitrophenol is followed at 405 nm (37°C) for 2 min. Two samples were spiked with saliva to ensure that it can be detected by our method. In all samples, no amylase was detected using the method described, suggesting no contamination of breath condensate with saliva. The samples that were spiked with saliva, showed levels of >3,000 IU salivary amylase.

H₂O₂ concentration was determined by an enzymatic assay as previously described. Briefly, 250 μL of 420 μM 3’, 5,5’-tetrathylbenzimidazolone (dissolved in 0.42 mol/L citrate buffer, pH 3.8) and 10 μL of 52.5 U/mL of horseradish peroxidase (HRP; Sigma Chemicals; St Louis, MO) were reacted with 250 μL of the condensate for 20 min at room temperature. Subsequently, the mixture was acidified to a pH of 1 with 10 μL of 18 N sulfuric acid. The reaction product was quantitated at the absorbency of 450 nm using a double-beam spectrophotometer (Uvicon 940; Kontron Instruments; Zurich, Switzerland). A standard curve was performed for the assay, with limit of determination of 0.1 μM H₂O₂. The investigator who performed the H₂O₂ measurements was blinded to the clinical and functional status of the subjects as well as to the results of sputum inflammatory cells.

**Sputum Induction and Processing**

Sputum was induced by the inhalation of an aerosol 3.5% hypertonic saline solution generated by an ultrasonic nebulizer (model 2696; DeVilbiss; Somerset, PA) with modifications to improve its safety. At least 2 mL of sputum was collected into a sterile container. The sample from the first cough was discarded, as this is heavily contaminated with squamous epithelial cells.
An adequate sample was defined if the number of squamous epithelial cells was <50\% of the total number of inflammatory cells.\(^{16}\) Cytospin slides were prepared and stained with May-Grunwald-Giemsa. The person who performed the differential cell counts was not aware of the clinical and functional status of the patients, as well as of the expired breath condensate measurements. Two slides were used for counting, and at least 300 inflammatory cells were counted for each slide.

Statistical Analysis

Data concerning characteristics of the subjects are shown as mean (SD) and range. Data concerning the comparisons between the various parameters in the study groups are given as mean with 95\% confidence intervals (CIs) for the differences. Parameters from patients and control subjects, as well as from all the subgroups, were compared using the Student’s t test. Spearman’s rank correlation coefficient was used to investigate the relation between the various parameters. A p value < 0.05 was considered significant.

RESULTS

Repeated measurements on 2 consecutive days showed a mean (SD) within-subject difference of 0.07 (0.04) μM for patients and 0.03 (0.02) μM for normal subjects. The stability of \(\text{H}_2\text{O}_2\) in frozen samples showed no significant difference among the four measurements: 0.62 (0.41) μM after 2 days, 0.64 (0.4) μM after 1 week, 0.6 (0.4) μM after 2 weeks, and 0.6 (0.4) μM after 3 weeks (p = 0.55).

Mean \(\text{H}_2\text{O}_2\) levels were significantly increased in patients with bronchiectasis compared to normal subjects: 1.1 μM (95\% CI, 0.87 to 1.29 μM) vs 0.3 μM (95\% CI, 0.19 to 0.36 μM), respectively (p < 0.0001; Fig 1, top). Patients treated with ICS had similar values than those not receiving ICS: 1.15 μM (95\% CI, 0.81 to 1.5 μM) vs 1.02 μM (95\% CI, 0.73 to 1.3 μM), respectively (p = 0.55; Fig 1, top). Patients with \(P\) aeruginosa colonization had significantly higher levels of \(\text{H}_2\text{O}_2\) compared to patients without \(P\) aeruginosa colonization: 1.6 μM (95\% CI, 1.25 to 1.92 μM) vs 0.8 μM (95\% CI, 0.64 to 1.01 μM), respectively (p < 0.05; Fig 1, top). \(\text{H}_2\text{O}_2\) levels were significantly increased in patients receiving long-term oral antibiotic treatment compared to those not receiving any antibiotics: 1.3 μM (95\% CI, 0.95 to 1.7 μM) vs 0.9 μM (95\% CI, 0.67 to 1.16 μM), respectively (p < 0.05). The total cell count was significantly higher in patients with bronchiectasis compared to normal subjects: 3.7 × 10⁶/mL (95\% CI, 1.6 to 3.9 × 10⁶/mL) vs 2.1 × 10⁶/mL (95\% CI, 1.4 to 2.3 × 10⁶/mL) respectively (p < 0.001). The percentage of neutrophils obtained from induced sputum was significantly higher in patients with bronchiectasis than in normal subjects: 59\% (95\% CI, 54 to 63\%) vs 27\% (95\% CI, 24 to 36\%), respectively (p < 0.0001; Fig 1, bottom). Sputum macrophage levels were significantly lower in patients than in normal subjects: 40\% (95\% CI, 32 to 53\%) vs 72\% (95\% CI, 65 to 84\%), respectively (p < 0.0001; Fig 1, bottom).

Correlations are summarized in Table 2. There was a significant positive correlation between \(\text{H}_2\text{O}_2\) concentration in exhaled breath condensate and either the percentage of sputum neutrophils or the HRCT scoring scale system (r = 0.9, p < 0.0001; and r = 0.66, p < 0.0001, respectively; Fig 2, top left, A, and top right, B). A significant negative correlation was observed between FEV\(_1\) percent predicted and \(\text{H}_2\text{O}_2\) concentration in exhaled breath.

![Figure 1. Top: \(\text{H}_2\text{O}_2\) concentrations in expired-breath condensate of patients with bronchiectasis (All; n = 30; closed circles), inhaled corticosteroid-treated patients (ICS+; n = 14; closed squares), inhaled corticosteroid-naïve patients (ICS−; n = 16; open squares), patients with \(P\) aeruginosa colonization (PA+; n = 10; closed triangles), patients without \(P\) aeruginosa colonization (PA−; n = 20; open triangles), and normal subjects (Normals; n = 15; open circles). Each symbol represents one individual. The concentration of \(\text{H}_2\text{O}_2\) was significantly higher in patients with bronchiectasis compared to normal subjects (p < 0.0001). Patients with \(P\) aeruginosa colonization had significantly higher values compared to patients without \(P\) aeruginosa colonization (p < 0.05). Horizontal bars represent mean values. Bottom: Percentage of differential cell counts in induced sputum in patients with bronchiectasis and in normal subjects. * = Statistically significantly higher percentage of neutrophils in induced sputum of patients with bronchiectasis compared to normal subjects (p < 0.0001). ** = Statistically significant lower percentage of macrophages in induced sputum of patients with bronchiectasis compared to normal subjects (p < 0.0001). Data are shown as mean (SD).](image-url)
condensate (\(r = -0.42, p < 0.05\); Fig 2, bottom left, C). Finally, a significant positive correlation was observed between \(\text{H}_2\text{O}_2\) and the bronchiectasis severity score (BSS) \([r = 0.43, p < 0.05]; \text{Fig} 2, \text{bottom} \text{right, D}\). A negative correlation was found between FEV1 percent predicted and either the percentage of sputum neutrophils or the HRCT scoring scale system \([r = -0.39, p < 0.05]; \text{and} \quad r = -0.48, p < 0.05\), respectively). A significant positive correlation was found between percent number of sputum neutrophils and HRCT scoring scale system \([r = 0.60, p < 0.001]\). No correlation was found between \(\text{H}_2\text{O}_2\) and the percentage of macrophages in induced sputum \([r = 0.14, p = 0.54]\). There was a significant positive correlation between BSS and either the percentage of sputum neutrophils or the HRCT scoring scale system \([r = -0.67, p < 0.001]; \text{and} \quad r = 0.40, p < 0.05\), respectively). No correlation was

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**Table 2**—Associations Between \(\text{H}_2\text{O}_2\) Concentration and the Main Clinical, Inflammatory, and Functional Parameters in Patients With Bronchiectasis*  

<table>
<thead>
<tr>
<th>Variables</th>
<th>(\text{H}_2\text{O}_2)</th>
<th>HRCT</th>
<th>FEV1 % Predicted</th>
<th>BSS</th>
<th>Percentage of Neutrophils</th>
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<tbody>
<tr>
<td>Neutrophils %</td>
<td>0.9</td>
<td>(&lt; 0.0001)†</td>
<td>0.6</td>
<td>(&lt; 0.0001)†</td>
<td>-0.39</td>
</tr>
<tr>
<td>HRCT</td>
<td>0.66</td>
<td>(&lt; 0.0001)†</td>
<td>-0.48</td>
<td>(&lt; 0.05)†</td>
<td>0.4</td>
</tr>
<tr>
<td>FEV1 % predicted</td>
<td>-0.42</td>
<td>(&lt; 0.05)†</td>
<td>-0.48</td>
<td>(&lt; 0.05)†</td>
<td>-0.21</td>
</tr>
<tr>
<td>BSS</td>
<td>0.43</td>
<td>(&lt; 0.05)†</td>
<td>0.4</td>
<td>(&lt; 0.05)†</td>
<td>-0.42</td>
</tr>
</tbody>
</table>

*Correlations were conducted using the Spearman’s rank correlation coefficient.
†Significant correlation.

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**Figure 2**. Correlation between \(\text{H}_2\text{O}_2\) in expired-breath condensate and (top left, A) percentage of neutrophils in induced sputum \((r = 0.9, p < 0.0001)\), (top right, B) HRCT scoring scale system \((r = 0.66, p < 0.0001)\), (bottom left, C) FEV1 percent predicted \((r = -0.42, p < 0.05)\), and (bottom right, D) BSS \((r = 0.43, p < 0.05)\). Closed squares indicate individual data points; pred = predicted.
observed between BSS and FEV1, percent predicted. FVC percent predicted, duration of disease, and number of infective exacerbations in the previous year did not correlate with the any of the variables tested.

**Discussion**

We have demonstrated that patients with bronchiectasis showed elevated levels of exhaled H2O2, which were significantly correlated with the neutrophil differential counts in induced sputum, extent of disease, lung function impairment, and disease severity. We interpret these findings as confirmation that oxidative stress is increased in the airways of patients with bronchiectasis, and reflects the severity of the disease as well as the inflammatory process.

The concentration of H2O2 measured in exhaled-breath condensate may be considered as the net result of production vs scavenging. Compared with the cellular antioxidant scavenging systems, the extracellular space and airway have significantly less ability to scavenge reactive oxygen species. Thus, the airway inflammation that occurs in bronchiectasis and other acute or chronic inflammatory processes involving the respiratory tract may be particularly likely to induce increased oxidant production that is detectable by increased H2O2 in expired breath. Activation of inflammatory cells, including neutrophils, eosinophils, and macrophages, induces a respiratory burst, resulting in marked production of reactive oxygen species, including H2O2. However, the precise role of ROS in the pathophysiology of inflammatory process remains unclear due to the difficulty in identifying which cells produce ROS in the inflamed lung and bronchi in each disease, as well as to specify the activated state of the abovementioned cells. Therefore, the major problem is to determine a strong relationship between oxidant production and the development of an inflammatory reaction. Airway assessments performed by bronchoscopy biopsy and lung lavage have suggested that inflammation contributes to the development of bronchiectasis. Most observations dealing with inflammatory cells in bronchiectasis have focused on neutrophils that undoubtedly impact on the inflammatory process and may alter the oxidant-antioxidant balance. Similar results were observed in our study using a noninvasive method, such as sputum induction. The high percentage of neutrophils observed in this study, the strong correlation with H2O2 concentration, and the lack of correlation between macrophages and H2O2 concentration lead to the plausible explanation that in patients with bronchiectasis, neutrophils express the main source of H2O2 production, while macrophages seem not to be the cells that generate H2O2. These findings are partially supported by previous observations, where accumulation of neutrophils in bronchiectatic airways is associated with airways damage. Polymorphonuclear leukocytes release several molecules that mediate this damage, particularly proteases and oxidants. This means that neutrophilic inflammation in the bronchiectatic airways is an endogenous source of oxidants leading to proteolytic and cellular damage and thereby contributes to oxidant stress.

In patients with bronchiectasis, a correlation between the extent of the disease and the degree of lung function impairment has already been shown. Furthermore, the degree of lung function impairment and the number of neutrophils recovered from lung lavage appeared to be strongly correlated in most patients. These earlier observations lead to the plausible explanation that lung function impairment expresses both the degree of neutrophilic inflammation in the airway lumen and the extent of the disease. These data are partially confirmed by our study, which also showed a strong positive correlation between neutrophil differential counts in induced sputum and disease severity. The last observation is the first, to our knowledge, to give evidence that the inflammatory process in the airway of patients with bronchiectasis is strongly associated with both the severity and extent of the disease. Taking all these points into consideration and based on previous observations where early knowledge of these parameters could be beneficial for the monitoring and treatment of the disease, H2O2 concentration seems to be an appropriate way to detect and follow up the course of these features. However, our study was designed as cross-sectional and could not strongly support the above-mentioned statements, which would require prospective controlled longitudinal data.

The present cross-sectional study cannot demonstrate a causal relationship between ICS usage, long-term oral antibiotic treatment, and H2O2 concentration. In fact, patients receiving ICS had similar values with steroid-naïve patients, while patients receiving long-term oral antibiotics consistently showed the highest values. Prospective, controlled studies are needed for that purpose. However, our findings are similar to those previously reported, where steroids have failed to alter H2O2 production from polymorphonuclear leukocytes, and antibiotics reduced effectively the amount of inflammation during an exacerbation but they are inadequate in dealing with persistent airway inflammation. The finding that patients receiving long-term oral antibiotic treatment had the highest H2O2 values may be due to the fact that patients with the most severe infections were the only patients receiving antibiotic...
therapy. The results of our study and the findings of others suggest that *P. aeruginosa* colonization in patients with bronchiectasis is associated with more extensive disease, more severe disease, and greater lung function impairment. However, it remains unclear whether *P. aeruginosa* is the cause or the result of advanced disease. We additionally showed that chronic colonization with *P. aeruginosa* may have a causal role in the oxidant-mediated lung injury. This is partially supported by previous observations where bacterial products released from *P. aeruginosa* impair ciliary activity and lead to persistent inflammation by a pathway that involves neutrophils and is mediated by ROIs, which are not inhibited by antioxidants.

In summary, exhaled H₂O₂, an index of oxidative stress, may be an indirect index of neutrophilic inflammation, lung function impairment, and severity and extension of disease. As collection of breath condensate is simple and noninvasive, it may be useful in patients with severe disease who may not tolerate more invasive investigations. If certain oxygen species contribute to airway inflammation in patients with bronchiectasis, then oxygen radical scavengers or antioxidants could play a critical role in treating the disease. However, no justification exists for this treatment, and further studies are needed to confirm this hypothesis.

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