

Exhaled eicosanoids and biomarkers of oxidative stress in exacerbation of chronic obstructive pulmonary disease

Adam Antczak, Maciej Ciebiada, Tadeusz Pietras, Wojciech J. Piotrowski, Zofia Kurmanowska, Paweł Górski

Department of Pneumology and Allergy, Medical University of Lodz, Poland

Submitted: 21 July 2010

Accepted: 30 November 2010

Arch Med Sci 2012; 8, 2: 277-285

DOI: 10.5114/aoms.2012.28555

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Corresponding author:

Maciej Ciebiada MD, PhD
Department of Pneumology
and Allergy
Medical University of Lodz
22 Kopcinskiego
90-153 Lodz, Poland
Phone: 48 42 678-21-29,
48 42 677 66 99
Fax: 48 42 678 21 29
E-mail:
maciej_ciebiada@op.pl

Abstract

Introduction: Eicosanoids and oxidants play an important role in inflammation, but their role in chronic obstructive pulmonary disease (COPD) is uncertain. In this study we hypothesized that levels of exhaled leukotrienes, prostaglandins and biomarkers of oxidative stress are increased in infectious exacerbations of COPD and that they decrease after antibiotic therapy.

Material and methods: Cysteinyl-leukotrienes (LTs), leukotriene B₄ (LTB₄), prostaglandin E₄, hydrogen peroxide (H₂O₂) and 8-isoprostane were measured in exhaled breath condensate (EBC) in 16 COPD patients with infectious exacerbations (mean age 64 ±12 years, 13 male) on day 1, during antibiotic therapy (days 2-4), 2-4 days after therapy and at a follow-up visit when stable (21-28 days after therapy).

Results: There was a significant fall in concentration of cys-LTs, LTB₄ and 8-isoprostane at visit 3 compared to day 1 (cys-LTs: 196.5 ±38.4 pg/ml vs. 50.1 ±8.2 pg/ml, $p < 0.002$; LTB₄: 153.6 ±25.5 pg/ml vs. 71.9 ±11.3 pg/ml, $p < 0.05$; 8-isoprostane: 121.4 ±14.6 pg/ml vs. 56.1 ±5.2 pg/ml, $p < 0.03$, respectively). Exhaled H₂O₂ was higher on day 1 compared to that at visits 2 and 3 (0.74 ±0.046 μM vs. 0.52 ±0.028 μM and 0.35 ±0.029 μM, $p < 0.01$ and $p < 0.01$, respectively). Exhaled PGE₂ levels did not change during exacerbations of COPD. Exhaled eicosanoids and H₂O₂ in EBC measured at the follow-up visit (stable COPD) were significantly higher compared to those from healthy subjects.

Conclusions: We conclude that eicosanoids and oxidants are increased in infectious exacerbations of COPD. They are also elevated in the airways of stable COPD patients compared to healthy subjects.

Key words: chronic obstructive pulmonary disease, exhaled eicosanoids, 8-isoprostane, exhaled breath condensate.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by the progressive development of airflow limitation associated with chronic inflammation [1]. The most important pathological changes are found within the small airways and are characterized by an increased number of macrophages, neutrophils and CD8+ T cells, features which are also found in bronchial biopsies [1]. There is also an increase in macrophages and neutrophils in bronchoalveolar lavage fluid and induced sputum [2]. Furthermore, increased neutrophils in the airways are related to the rate of progression of airway obstruction [3]. Smoking, which is the most important risk factor, increases neutrophil recruitment to the lungs [4], possibly by stimulating bronchial

epithelium and macrophages to produce the neutrophil chemoattractant interleukin-8 (IL-8) [5]. Moreover, levels of IL-8 and leukotriene B₄ (LTB₄), which are also chemotactic for neutrophils, are increased in the sputum of patients with COPD [6]. In contrast to asthma, eosinophils are not increased except during exacerbations [7].

There is a complex interaction between cells and mediators in COPD, resulting in progressive changes in small airways and parenchymal damage [1]. It is postulated that macrophages play a critical role, since they are 5 to 10 times increased and when activated are capable of producing all the pathological changes of COPD. They are activated by cigarette smoke to release neutrophil chemoattractants and other mediators of inflammation including eicosanoids [5].

There is overwhelming evidence that oxidative stress may have an important role in COPD [8]. Hydrogen peroxide (H₂O₂) levels in exhaled breath condensate of patients with COPD are increased, particularly during exacerbations [9]. Plasma antioxidant capacity is decreased in patients with acute exacerbations of COPD [10]. It has also been clearly shown that oxidative overburden leads to lipid peroxidation in the airway of patients with COPD as reflected by greater exhaled ethane and 8-isoprostane levels compared to healthy subjects [11].

Eicosanoids are important inflammatory mediators in asthma, but their role in COPD remains unclear. The LTB₄ was detected in nasal lavage [12], sputum and BAL fluid in patients with stable COPD and levels of LTB₄ in sputum and BAL correlate with other markers of inflammation such as myeloperoxidase and elastase activity [13]. Moreover, serum LTB₄ levels in patients with COPD are higher than in healthy subjects [14]. In contrast, Piperno *et al.* demonstrated that LTC₄ but not LTB₄ is detectable in the plasma of patients with COPD [15]. However, plasma levels of leukotrienes are difficult to interpret due to release from inflammatory cells and metabolism. Cys-LTs are potent bronchoconstrictors and play an important role in asthma, particularly in the aspirin-sensitive type [16]. They have also been detected in the sputum of patients with chronic bronchitis [17]. The prostaglandin E₂ (PGE₂) relaxes airway smooth muscles and exerts potent anti-inflammatory activity [18]. It is postulated that the failure in a PGE₂-braking mechanism with increased sensitivity to inhibition by NSAIDs contributes to the overproduction of cys-LTs in aspirin-induced asthmatic patients [16].

Exhaled breath condensate (EBC) provides a non-invasive and easy-to-perform means of looking at the local inflammatory process in the airways, without the need to undertake invasive procedures, such as bronchoscopy. Most of the studies to explore the role of eicosanoids in COPD have used invasive techniques or the assessment of media-

tors and their metabolites in plasma or urine. Concentrations of eicosanoids in these biological fluids are more likely to reflect systemic, rather than respiratory tract inflammation. An increasing body of evidence suggests that EBC is a useful way to monitor markers of inflammation and oxidative stress in various respiratory tract diseases.

Exhaled cys-LTs and PGE₂ are markers of inflammation in the airways. The LTB₄ plays an important role in neutrophil recruitment and activation at the site of inflammation. 8-isoprostane and H₂O₂ are biomarkers of oxidative stress in breath condensate in inflammatory airway diseases such as asthma, COPD, and cystic fibrosis [19].

Accordingly, the aim of this study was to determine eicosanoids, 8-isoprostane and H₂O₂ at various time points in COPD patients hospitalized for an infectious exacerbation of COPD and treated with antibiotics, and to investigate the relationship between these inflammatory markers and clinical variables (airflow limitation and respiratory failure).

Material and methods

Study population

Sixteen patients with severe infectious exacerbations of COPD (mean age 64 ±12 years, 13 male), and 13 healthy age-matched controls (mean age 57 ±19 years, 10 male) were recruited for this study (Table I). Patients from the control group had no history of any respiratory disease or respiratory infection, they were non-smokers and they did not suffer from any other disease that could exclude them from the study. The COPD was diagnosed by a history of cough and sputum production for more than 2 consecutive years and for most days in a consecutive 3-month period and spirometry criteria according to GOLD standards [1]. Exacerbation of COPD was defined as "an event in the natural course of the disease characterized by a change in the patient's baseline dyspnoea, cough and/or sputum that is beyond normal day-to-day variations, is acute in onset and may warrant a change in regular medication" [1]. The severity of exacerbation was assessed on the basis of patients' history before the exacerbation (duration of worsening, previous hospitalisations, previous treatment), comorbidities, symptoms, physical diagnosis, and measurements of arterial blood gases [1]. In this study exacerbation of COPD was characterized by increased purulent sputum together with increased dyspnoea and cough. Other inclusion criteria were forced expiratory volume in 1 s (FEV₁) > 50% predicted, at least 3 episodes of exacerbation of COPD in the past year requiring treatment with an antibiotic, and age ≥ 40 years. Exclusion criteria were a known or suspected hypersensitivity to cephalosporins or macrolides, cystic fibrosis, bronchiectasis or active pulmonary malignancies, immunocompromised patients, and patients

Table I. Characteristics of study population

No	Age [years]	Body temperature [°C]	WBC	Neutrophil absolute	Current smoking status [cigarettes/day]	Cumulative smoking habit [pack years]	Pathogen	FEV ₁ [% pred.]	FEV ₁ /FVC [%]
1. ♂	55	38.4	8.4	5.43	Non-smoking	30	<i>Staphylococcus aureus</i> , <i>Streptococcus pneumoniae</i>	69.5	65
2. ♀	70	36.6	12.3	8.08	20	50	<i>S. aureus</i> , <i>S. pneumoniae</i>	56.9	60
3. ♂	58	38.2	9.2	6.6	10	15	<i>S. pneumoniae</i>	74.3	69
4. ♀	76	38.0	10.8	8.55	20	30	<i>S. pneumoniae</i>	55.3	58
5. ♂	60	37.8	13.5	10.52	20	45	No pathogen isolated	69.9	67
6. ♂	58	36.0	7.6	5.29	20	20	<i>Candida</i> sp.	72.1	66
7. ♂	66	36.8	6.9	5.33	25	40	<i>S. pneumoniae</i>	49.9	57
8. ♂	56	38.6	7.8	4.77	20	30	<i>S. aureus</i>	68.6	63
9. ♂	60	38.3	11.2	7.85	40	120	<i>Klebsiella pneumoniae</i>	54.9	60
10. ♂	77	38.0	11.6	8.83	40	90	<i>S. aureus</i>	66.3	67
11. ♂	43	38.0	18.7	17.15	Non-smoking	45	<i>S. aureus</i>	45.7	50
12. ♂	56	37.2	16.1	14.59	20	60	<i>Proteus vulgaris</i>	79.9	68
13. ♂	57	38.0	8.2	5.64	20	30	<i>S. aureus</i> , <i>S. pneumoniae</i>	59.9	63
14. ♂	77	38.0	4.7	3.92	15	37.5	<i>S. aureus</i> , <i>S. pneumoniae</i> , <i>Pseudomonas aeruginosa</i>	62	60
15. ♂	71	37.6	8.7	5.09	20	40	<i>S. pneumoniae</i>	52.5	59
16. ♀	69	36.4	12.1	10.2	Non-smoking	35	<i>Escherichia coli</i>	53.6	61

receiving systemic steroids. Due to exacerbation all patients were treated with inhaled steroids (1000 µg of budesonide or equivalent as an alternative to systemic steroids [1]) and they had been immediately admitted to the hospital. They continued steroid therapy through the entire hospitalisation (inhaled steroids with or without systemic steroids at a dose of < 30 mg per day of prednisone or equivalent up to 7 days). Bronchodilators were allowed in the study including inhaled or nebulized ipratropium bromide and albuterol. Long-acting β₂-agonists were used (formoterol up to 54 µg/day). All subjects completed an informed written consent form, and the study was approved by the Ethics Committee of the Medical University of Lodz.

Study design

This was a randomized, open label, controlled, single-centre study to compare changes in exhaled eicosanoids and H₂O₂ in patients with exacerbation

of COPD treated for at least 7 days with oral roxithromycin (7 patients, 150 mg, twice daily) or oral cefuroxime axetil (9 patients, 500 mg twice daily) randomly allocated (1 : 1) to the patients. Average duration of treatment was 7.3 ± 0.6 days. Patients were assessed, exhaled breath condensate was obtained, and spirometry and arterial blood gases measurements were done at the baseline visit (visit 1), during therapy (days 2-4), at the end of therapy (2-4 days after therapy) and at a follow-up visit (21-28 days after therapy) (Table II). A sputum specimen for bacteriological evaluation (gram staining, culture and susceptibility testing) was collected at visit 1.

Exhaled breath condensate

Breath condensate samples were obtained using a condensing tubing system, as previously described [20]. Briefly, patients were asked to breathe through the collection tube for 15 min. The collection part of the tube was covered with dry ice.

Table II. Exhaled eicosanoids and H₂O₂ in stable COPD patients and healthy subjects

Parameter	COPD (n = 16)	Healthy (n = 11)	Value of p
Cys-LTs [pg/ml]	41.7 ±6.83	22.4 ±3.25	< 0.02
LTB ₄ [pg/ml]	75.8 ±10.71	40.1 ±3.41	< 0.003
PGE ₂ [pg/ml]	41.1 ±2.09	20.4 ±5.21	< 0.004
8-Isoprostane [pg/ml]	60.9 ±3.84	31.3 ±4.27	< 0.001
H ₂ O ₂ [mM]	0.35 ±0.029	0.17 ±0.28	< 0.001

Means ± standard error of the mean are shown

The temperature in the tube vicinity ranged from –43°C to –32°C and allowed collection of all vapours and droplets present in the exhaled air. At the end of the collection, the tube was removed from the container and 2–6 ml aliquots of condensate were transferred to Eppendorf tubes and stored at –80°C for not more than 4 weeks. Due to the impact of cigarette smoke on oxidants and eicosanoids present in exhaled breath, our patients refrained from smoking for at least 12 h before measurement. It was easy because they were in exacerbation of COPD and most of them did not even smoke due to their symptoms.

Measurement of leukotrienes

Cys-LTs concentration in breath condensate was measured using a specific enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI). The antiserum used in this assay has 100% cross-reactivity with LTC₄ and LTD₄, 67% with LTE₄, and < 0.01% each with 15-HETE, 12-HETE, LTB₄, PGF₂. The detection limit of the assay is 13 pg/ml. This kit has been used to measure concentrations of cys-LTs in rat and human urine, plasma, and BAL fluid [12, 21].

The LTB₄ was measured using the EIA kit (Cayman Chemical) in breath condensate. The antiserum used in this assay has 100% cross-reactivity with LTB₄, 39% with 6-trans LTB₄, and < 0.01% each with LTC₄, LTE₄, LTD₄, and LTF₄, and a detection limit of 4.43 pg/ml.

Measurement of immunoreactive 8-isoprostane

8-isoprostane concentration in breath condensate was measured using an EIA kit (Cayman Chemical). The antiserum used in this assay has 100% cross-reactivity with 8-epi-PGF_{2α}, 0.2% each with PGF_{2α}, PGF_{3α}, PGE₁, PGE₂, and 0.1% with 6-keto-PGF_{1α}. The detection limit of the assay is 4 pg/ml.

Measurement of immunoreactive prostaglandin E₂

The PGE₂ concentration in breath condensate was measured using a specific EIA kit (Cayman

Chemical). The antiserum used in this assay has 100% cross-reactivity with PGE₂, 43% with PGE₃, 18.7% with PGE₁, and 0.1% each with PGF_{2α}, PGA₁, PGA₂, and the detection limit at 4°C is 15 pg/ml. For all measurements 50 μl aliquots of the condensate were used.

Detection of hydrogen peroxide

The H₂O₂ was detected according to the method of Ruch [22] and readings were expressed in μM using the regression equation $Y = (X - X_0)0.0676$ (where Y = micromoles of H₂O₂ per litre of EBC; X = intensity of emission; X₀ = intensity of emission given by reference sample receiving distilled water instead of EBC). For all measurements 600 μl aliquots of condensate mixed with horseradish peroxidase solution (1 U/ml) were used. The H₂O₂ concentration was determined spectrofluorimetrically using a Perkin Elmer Spectrometer (Norwalk, USA).

Spirometry

Post-bronchodilation spirometry was performed before the start of the treatment with inhaled steroids in the outpatient clinic or in the hospital (visit 1), at visit 2 and the post-therapy visit (visit 3) and at the follow-up visit with a computer-assisted spirometer (Lung Test 1000, MES Dymek, Dabrowski SA, Krakow, Poland) according to standardized guidelines. Values were expressed as percentage of predicted values.

Arterial blood gases

A sample of arterialised capillary blood from the finger pulp was taken in all COPD patients and pO₂ and pCO₂ were assessed to determine the presence of respiratory failure.

Statistical analysis

Statistical analyses included a two-way analysis of variance (ANOVA). Repeated measures test (Friedman test) was used to compare groups. Linear regression analysis was used to assess the relationship between measured parameters. Readings below the method detection limit were arbitrarily assumed as half way between the detection limit and 0. All data are expressed as means ± standard error of the mean and significance was defined as a p value of < 0.05. Statistica 5.1 PL for Windows software (StatSoft Polska, Cracow, Poland) was used for analyses.

Results

Table II shows mean exhaled eicosanoid and H₂O₂ levels from stable COPD patients (as assessed at follow-up visit) and healthy control subjects. Mean volume of exhaled breath condensate was

2.22 ±0.31 ml at visit 1, 2.37 ±0.42 at visit 2, and 2.41 ±0.25 at visit 3 for COPD patients. Stable COPD patients had significantly higher levels of all eicosanoids compared to healthy subjects.

Leukotrienes

Significantly greater levels of exhaled cys-LTs were observed at visit 1 compared to those at visit 2 and the post-therapy visit (196.5 ±38.41 vs. 78.6 ±12.28 and 50.1 ±8.15 pg/ml, $p < 0.03$ and $p < 0.002$, respectively) and at the follow-up visit (41.7 ±6.83 pg/ml, $p < 0.002$) (Figure 1). There were significantly higher levels of exhaled LTB₄ at visit 1 compared to those at visit 3 but not during antibiotic therapy (153.6 ±25.51 vs. 132.0 ±29.31 and 71.9 ±11.25 pg/ml, $p < 0.03$ and $p < 0.05$, respectively). There was also a significant difference between levels of LTB₄ in exhaled breath condensate on day 1 and at the follow-up visit (75.8 ±10.71 pg/ml, $p < 0.03$). There were no differences between cephalosporin and macrolide treated groups.

Prostaglandin E₂

In contrast to changes in exhaled cys-LTs and LTB₄ levels, exhaled PGE₂ levels did not change during exacerbations of COPD (47.0 ±3.71 vs. 40.3 ±2.49 and 36.9 ±3.09 pg/ml, respectively, $p > 0.05$) (Figure 3) and remained higher even at follow-up compared to healthy subjects (41.0 ±2.09 vs. 20.4 ±5.21 pg/ml, $p < 0.004$). There were no differences in exhaled cys-LTs and LTB₄ concentration between cephalosporin and macrolide treated groups.

8-Isoprostane

8-isoprostane levels in expired breath condensate during exacerbation of COPD are shown in Figure 4. There were significantly greater levels of exhaled 8-isoprostane on day 1 compared to those during therapy and at post-therapy visits (121.4 ±14.59 vs. 92.4 ±6.28 and 56.1 ±5.15 pg/ml, $p < 0.05$ and $p < 0.03$, respectively) and at the follow-up visit (60.9 ±3.84 pg/ml). There was also a significant difference in 8-isoprostane levels between visits 2 and 3 ($p < 0.001$). There were no differences between cephalosporin and macrolide treated groups.

Hydrogen peroxide

As seen in Figure 5, there were significantly greater levels of exhaled H₂O₂ on day 1 compared to those during therapy and at post-therapy visits (0.74 ±0.046 vs. 0.52 ±0.028 and 0.35 ±0.029 μM, $p < 0.001$ and $p < 0.001$, respectively) and at the follow-up visit (0.33 ±0.022 μM, $p < 0.001$). There was also a significant difference in H₂O₂ levels between visits 2 and 3 ($p < 0.01$). There were no differences

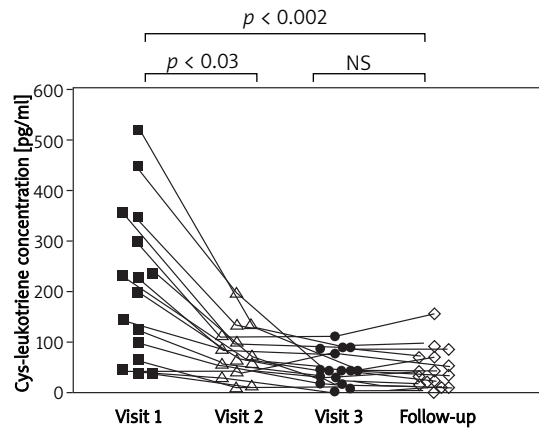


Figure 1. Cys-LTs in expired breath condensate of exacerbated COPD patients

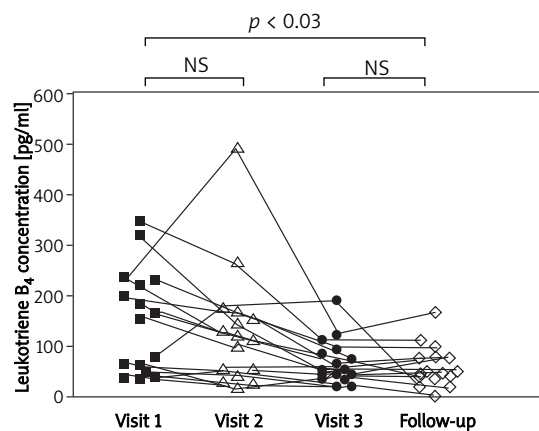


Figure 2. The LTB₄ in expired breath condensate of exacerbated COPD patients

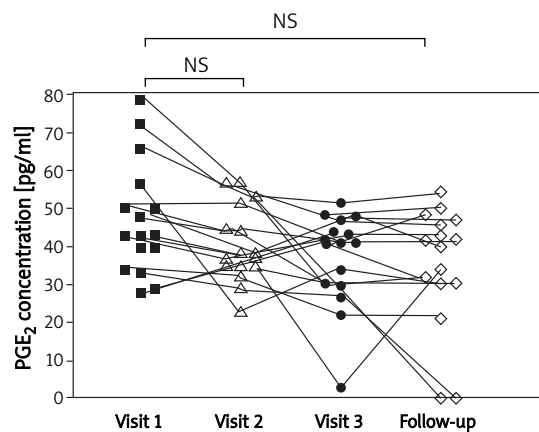


Figure 3. The PGE₂ in expired breath condensate of exacerbated COPD patients

between cephalosporin and macrolide treated groups. There was a positive correlation between exhaled 8-isoprostane and H₂O₂ levels at visit 1 ($r = 0.62$, $p < 0.05$) (Figure 6). There were no correlations between exhaled eicosanoids and H₂O₂ and clinical status, smoking status and pulmonary function tests at all visits.

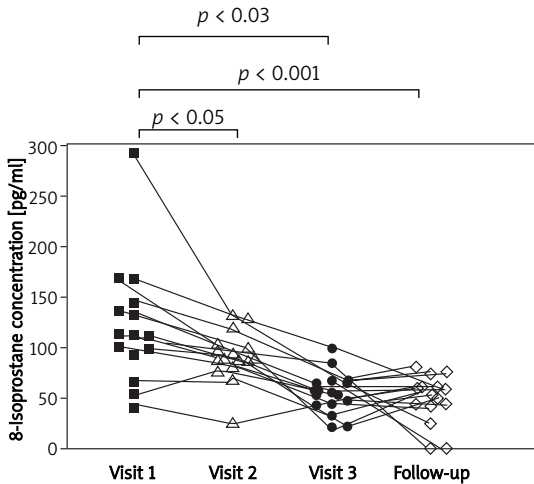


Figure 4. The 8-isoprostane levels in expired breath condensate of exacerbated COPD patients

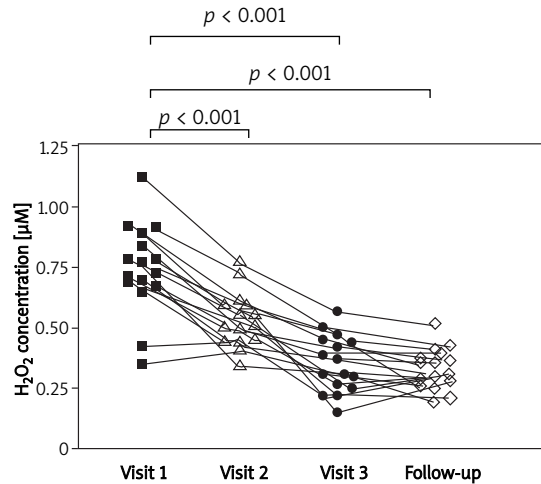


Figure 5. The H₂O₂ in expired breath condensate of exacerbated COPD patients

Pulmonary function tests and pO₂

Significantly lower FEV₁%pred. values were observed at visit 1 compared to those at visit 2 and the post-therapy visit (62 ± 2 vs. 69 ± 1 and 73

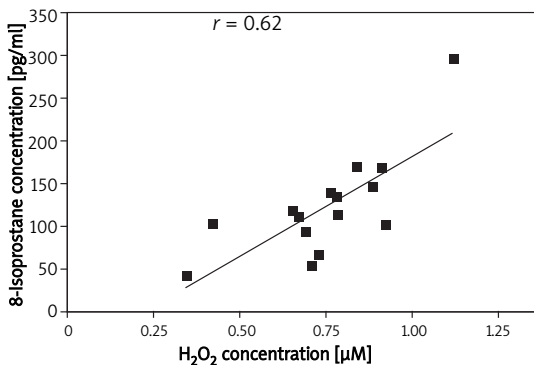


Figure 6. Positive correlation between 8-isoprostane and H₂O₂ levels in expired breath condensate of exacerbated COPD patients at visit 1 (day 1)

± 2 pg/ml, $p < 0.001$ and $p < 0.001$, respectively) and at the follow-up visit (71 ± 2 pg/ml, $p < 0.001$) (Figure 7 A). There were significantly lower pO₂ values at visit 1 compared to those at visit 3 and during antibiotic therapy (55 ± 1 vs. 73 ± 2 and 68 ± 2 mm Hg, $p < 0.001$ and $p < 0.001$, respectively). There was also a significant difference between pO₂ on day 1 and at the follow-up visit (74 ± 2 mm Hg, $p < 0.001$) (Figure 7 B). There were no differences between cephalosporin and macrolide treated groups.

Discussion

We demonstrated that eicosanoids (cys-LTs, LTB₄, 8-isoprostane) and H₂O₂, well known for their pro-inflammatory activity, are significantly elevated in patients during acute exacerbations of COPD and that they decrease during treatment, although they remain in higher concentrations in stable COPD patients compared to healthy subjects. Similarly, there are increased levels of exhaled PGE₂ in expired breath condensate from exacerbated COPD

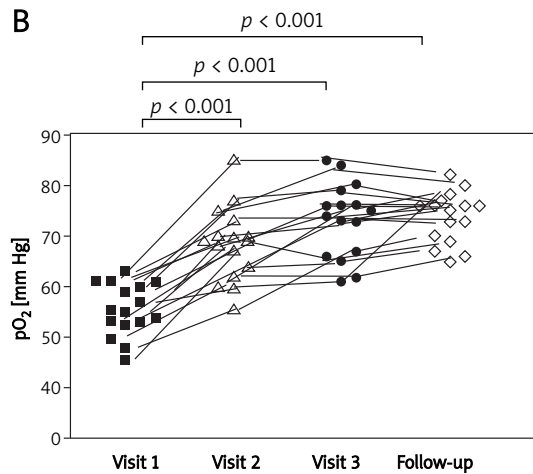
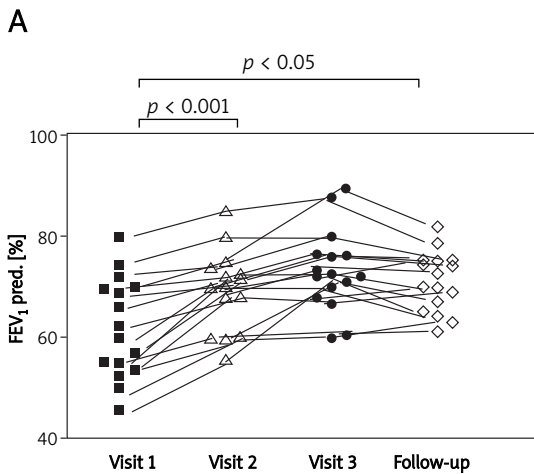


Figure 7. Changes of FEV₁ (A) and pO₂ (B) in patients with exacerbation of COPD

patients but they do not change in the course of treatment and are significantly higher than those from healthy subjects.

In this study comparative measurements of pro-inflammatory cys-LTs, LTB₄, 8-isoprostanes and H₂O₂ and anti-inflammatory (bronchoprotective) PGE₂ have been performed, using exhaled breath condensate from patients with bacterial exacerbations of COPD, and healthy controls. Exhaled breath condensate provides a non-invasive and low-tech means of looking at the local inflammatory process in the airways. It is very well tolerated by patients and no adverse effects have been reported. This technique has been used to study oxidative stress in COPD subjects [11] and an increasing body of evidence suggests that it is a useful way to monitor markers of inflammation and oxidative stress in various respiratory tract diseases, such as asthma, COPD and cryptogenic fibrosing alveolitis [23].

Altered metabolism of arachidonic acid may be one of the mechanisms of bronchoconstriction in COPD patients, particularly during exacerbation. Our study now demonstrates local overproduction of cys-LTs, LTB₄ and PGE₂ in the airways in COPD and is consistent with the study showing increased plasma levels of LTE₄ in acute exacerbations of COPD which decrease after steroid treatment [24]. Increased levels of LTB₄ have also been found in sputum from COPD patients with α 1-antitrypsin deficiency [6]. There are few data on the effects of antibiotic treatment on eicosanoid profiles and it can only be speculated that both cephalosporins and roxithromycin may reduce the number of activated cells that release pro-inflammatory cytokines in the airways by blocking their influx into the lung [25]. Roxithromycin has some immunoregulatory effects in chronic respiratory tract infection reflected by reducing levels of LTB₄ in epithelial lining fluid [25].

The fact that leukotrienes but also prostaglandins levels are increased during exacerbations suggests that there might be an increase in 5-lipoxygenase enzyme activity in COPD patients. Eosinophils are increased in bronchial mucosa in COPD during exacerbations compared to stable COPD and may be responsible for increased cys-LTs.

In contrast to elevated cys-LTs and LTB₄, PGE₂ levels in exhaled breath condensate of COPD patients are high even after treatment and at follow-up. It is possible that PGE₂ may play a bronchoprotective role. Moreover, PGE₂ may inhibit the release of LTB₄ from neutrophils in a concentration-dependent manner [26]. Our results clearly show that local levels of PGE₂ in the airways are increased in COPD compared with healthy subjects and this might be an adaptive mechanism to protect the airways from bronchoconstrictive agents which, as we show in this study, are increased even in clinically

stable subjects. Moreover, as PGE₂ levels did not change throughout the study it seems to be an argument that a decrease in the levels of other markers is not related to a decrease in the volume of droplets logically expected in exacerbated patients with increased secretions. If a changed volume of droplets produced in the airways was responsible for observed changes in mediators, the change would also be seen in all mediators including PGE₂.

8-Isoprostane, a stable prostaglandin-like arachidonate product formed on membrane phospholipids by the action of reactive oxygen species, is postulated to be a reliable biomarker of lipid peroxidation caused by oxygen reactive species and to represent a quantitative measure of oxidative stress *in vivo* [27]. The 8-isoprostane appears to reflect oxidative stress in breath condensate and is progressively increased with the severity of asthma, and its levels are particularly elevated in aspirin-induced asthma [28]. It is also reported to be increased in expired breath condensate in both stable and exacerbated COPD patients [19]. In this study we found further increases in the levels of exhaled 8-isoprostane in exacerbations of COPD patients. It has been well documented that an oxidant-antioxidant imbalance in favour of the latter is a hallmark of COPD. 8-isoprostane levels decreased during treatment, but remained higher in stable COPD patients compared to healthy subjects. It is not certain if 8-isoprostane is involved in the pathogenesis of COPD, but 8-isoprostane can cause contraction of human bronchi *in vitro* [29].

We also found an increase in H₂O₂ in exhaled breath condensate during acute exacerbations of COPD, which is in agreement with a previous study [9]. Furthermore, there is a correlation between exhaled H₂O₂ and 8-isoprostane levels, providing further evidence that oxidative stress is increased during exacerbation. The increased content of H₂O₂ and 8-isoprostane in expired breath condensate of COPD subjects is likely to be due to increased oxidant production and subsequent lipid peroxidation in the bronchial lining fluid. This is likely to be due to an increase in the number of activated inflammatory cells such as neutrophils and macrophages in the airways. Some healthy subjects also had measurable exhaled 8-isoprostane and H₂O₂, which is consistent with studies showing the peroxide-dependent spontaneous chemiluminescence of human breath and with our previous study in which 22% of non-smoking subjects revealed a detectable content of H₂O₂ in expired breath condensate [30].

Abrogation of oxidative stress and lipid peroxidation in exacerbations of COPD by antibiotics may be the effect of a reduction in the number and activation of inflammatory cells producing reactive oxygen species and eicosanoids.

However, we did not find any correlations between exhaled eicosanoids, H₂O₂ and 8-isoprostane and clinical status, smoking status and pulmonary function tests at all visits in COPD patients. Although there have been some trials that showed such correlations [31], there have been a few studies that could not find any significant association between concentration of inflammatory mediators and lung function impairment in COPD patients measured with FEV₁ [11, 31]. The lack of correlation in our study could be due to the low number of participants. Another explanation is that FEV₁ and inflammatory mediators are different markers of COPD. Concentration of exhaled mediators (i.e. H₂O₂, 8 isoprostane, leukotrienes) reflects oxidative stress while FEV₁ reflects calibre of airways, and it is possible that oxidative stress is present even without airflow limitation. The lack of correlation does not diminish the importance of the fact that the EBC mediators could be used in the study of inflammation in the airways of patients with COPD.

In conclusion, we have shown that there are increased levels of both pro-inflammatory and anti-inflammatory eicosanoids and also increased oxidative stress as reflected by increased exhaled 8-isoprostane and H₂O₂ during infectious exacerbations of COPD. Although reduced after antibiotic and anti-inflammatory treatment, eicosanoids and markers of oxidative stress remain in higher concentrations locally in the airways, as reflected by the increased levels in exhaled breath condensate of patients with stable COPD compared to normal subjects. These mediators may play a pathophysiological role in COPD and measurement of these markers in the exhaled air may provide a useful and non-invasive approach to study inflammation in the airways during exacerbations.

Acknowledgments

This study has been done with financial support from the Polish Research Committee (grant no. PBZ/KBN/004/CD/T-11/2000) and from the Medical University of Lodz no. 502-11-827.

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