

## CHRONIC OBSTRUCTIVE PULMONARY DISEASE

# Exhaled leukotrienes and prostaglandins in COPD

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**Background:** The role of eicosanoids, including leukotrienes (LTs) and prostaglandins (PGs), in chronic obstructive pulmonary disease (COPD) is uncertain. The aim of this study was to investigate whether eicosanoids are measurable in exhaled breath condensate (EBC), a non-invasive method of collecting airway secretions, in patients with stable mild to moderate COPD, and to show possible differences in their concentrations compared with control subjects.

**Methods:** LTB<sub>4</sub>, LTE<sub>4</sub>, PGE<sub>2</sub>, PGD<sub>2</sub>-methoxime, PGF<sub>2α</sub> and thromboxane B<sub>2</sub> (TxB<sub>2</sub>) were measured in EBC in 15 healthy ex-smokers, 20 steroid naïve patients with COPD who were ex-smokers, and in 25 patients with COPD who were ex-smokers and who were treated with inhaled corticosteroids. The study was of cross sectional design and all subjects were matched for age and smoking habit.

**Results:** LTB<sub>4</sub> and PGE<sub>2</sub> concentrations were increased in steroid naïve (LTB<sub>4</sub>: median 100.6 (range 73.5-145.0) pg/ml, p<0.001; PGE<sub>2</sub>: 98.0 (range 57.0-128.4) pg/ml, p<0.001) and steroid treated patients with COPD (LTB<sub>4</sub>: 99.0 (range 57.9-170.5) pg/ml, p<0.001; PGE<sub>2</sub>: 93.6 (range 52.8-157.0) pg/ml, p<0.001) compared with control subjects (LTB<sub>4</sub>: 38.1 (range 31.2-53.6) pg/ml; PGE<sub>2</sub>: 44.3 (range 30.2-52.1) pg/ml). Both groups of patients had similar concentrations of exhaled LTB<sub>4</sub> (p=0.43) and PGE<sub>2</sub> (p=0.59). When measurable, LTE<sub>4</sub> and PGD<sub>2</sub>-methoxime concentrations were similar in COPD patients and controls, whereas PGF<sub>2α</sub> concentrations were increased in the former. TxB<sub>2</sub>-L was undetectable in any of the subjects.

**Conclusions:** There is a selective increase in exhaled LTB<sub>4</sub> and PGE<sub>2</sub> in patients with COPD which may be relatively resistant to inhaled corticosteroid therapy.

The role of leukotrienes (LTs) and prostaglandins (PGs) in the pathophysiology of chronic obstructive pulmonary disease (COPD) is uncertain. LTB<sub>4</sub>, a potent neutrophil chemoattractant, and cysteinyl(cys)-LTs which cause bronchoconstriction and have proinflammatory effects are detectable in sputum in patients with chronic bronchitis.<sup>1,2</sup> The concentrations of LTB<sub>4</sub> in serum are increased in patients with COPD<sup>3</sup> and release of LTB<sub>4</sub> by alveolar macrophages is increased in α<sub>1</sub>-antitrypsin deficiency.<sup>4</sup> Sputum levels of PGE<sub>2</sub>, PGF<sub>2α</sub>, 6-oxo-PGF<sub>1α</sub>, and thromboxane (Tx)B<sub>2</sub> have been measured in patients with COPD but their effects are unknown.<sup>2</sup>

Most of the studies investigating the role of eicosanoids in COPD have used invasive techniques such as bronchoalveolar lavage<sup>5,6</sup> or have measured these compounds in plasma or urine remote from the site of production.<sup>3,7</sup> 8-Isoprostane, a

PGF<sub>2α</sub> analogue which reflects oxidative stress, is increased in exhaled breath condensate (EBC) in patients with COPD.<sup>8</sup> EBC is a completely non-invasive method of sampling secretions from the airways.<sup>9-11</sup> LTB<sub>4</sub> and LTE<sub>4</sub>, but not PGE<sub>2</sub>, are increased in EBC in steroid naïve asthmatic patients compared with healthy subjects.<sup>12</sup> The aim of the present study was to measure EBC concentrations of LTs and PGs in steroid naïve and steroid treated patients with stable COPD to identify possible selective profiles of eicosanoids in COPD.

## METHODS

### Subjects and study design

A random sample of 15 healthy subjects, 20 steroid naïve patients with COPD, and 25 patients with COPD who were treated with inhaled corticosteroids participated in the cross sectional study (table 1).

**Table 1** Characteristics of study subjects

	Healthy subjects (n=15)	Steroid naïve COPD patients (n=20)	Steroid treated COPD patients (n=25)†
Age (years)	60 (5)	65 (2)	67 (2)
F/M	8/7	11/9	13/12
Smoking	Ex	Ex	Ex
Pack years	>10	>10	>10
FEV <sub>1</sub> (l)	4.55 (0.29)	1.29 (0.27)*	1.41 (0.19)*
FVC (l)	4.72 (0.31)	2.41 (0.25)*	2.56 (0.15)*
FEV <sub>1</sub> (% pred)	95.1 (4.5)	52.4 (3.8)*	50.6 (4.3)*
FVC (% pred)	99.2 (3.7)	79.4 (3.3)*	74.9 (4.0)*
FEV <sub>1</sub> /FVC (%)	96.4 (2.3)	53.2 (2.8)*	55.1 (3.3)*
Atopy	No	No	No
Treatment			
Theophylline	No	4/20	5/25
β <sub>2</sub> agonists	No	14/20	15/25

Groups were frequency matched by age and smoking habit. Data are expressed as means (SE).

COPD=chronic obstructive pulmonary disease; FEV<sub>1</sub>=forced expiratory volume in 1 second; FVC=forced vital capacity.

\*p<0.01 compared with healthy subjects.

†Inhaled corticosteroids: beclomethasone dipropionate (dose range 0.2-1 mg/day), budesonide (dose range 0.4-0.8 mg/day), and fluticasone propionate (dose range 0.75-2 mg/day).

Subjects attended on one occasion for clinical examination, spirometry, and EBC collection. The diagnosis of COPD was based on the Global Initiative for Obstructive Lung Disease (GOLD) guidelines.<sup>13</sup> All patients had moderate COPD<sup>13</sup> and were clinically stable with forced expiratory volume in 1 second ( $FEV_1$ ) <80% predicted and  $FEV_1$ /forced vital capacity ( $FEV_1/FVC$ ) ratio <70% which did not change markedly over 8 weeks. Patients with other respiratory or systemic diseases were excluded from the study. All study subjects were ex-smokers and had stopped smoking for at least 1 year. Smoking status was checked by measuring urinary cotinine levels which were <10 ng/ml in all study subjects. None of the steroid naïve COPD patients had received corticosteroids in the previous 4 weeks. Inhaled  $\beta$  adrenergic agonists and theophylline were also used in some patients (table 1).

Repeatability for eicosanoid measurements was assessed in 25 patients with COPD. A second EBC sample was collected within 7 days of the first sample.

The study was approved by the ethics committee of the Royal Brompton Hospital and Harefield Trust and informed consent was obtained from all subjects.

### Pulmonary function

Spirometric tests were performed using a dry spirometer (Vitalograph Ltd, Buckingham, UK) and the best value of the three manoeuvres was expressed as an absolute value (litres) and as a percentage of the predicted value.

### Measurement of exhaled eicosanoids

EBC samples were collected using a commercially available condensing chamber, as previously described (Ecoscreen; Jaeger, Hoechberg, Germany).<sup>8</sup> Subjects breathed tidally through a mouthpiece connected to the condenser for 15 minutes.

$LTB_4$ ,  $LTE_4$ ,  $PGF_{2\alpha}$ ,  $TxB_2$ , and  $PGD_2$ -methoxime (MOX), a stable derivative of  $PGD_2$ , were measured by enzyme immunoassays (Cayman Chemical, Ann Arbor, MI, USA).  $PGE_2$  concentrations were measured by a radioimmunoassay (RIA).<sup>14</sup>

The intra-assay and inter-assay coefficients of variability of the eicosanoid assays were within 10% and 15%, respectively.  $LTB_4$  and  $PGE_2$  measurements in EBC were qualitatively validated by reverse phase high performance liquid chromatography.<sup>15,16</sup> The values of  $LTE_4$ ,  $PGF_{2\alpha}$ , and  $TxB_2$  were referred to as eicosanoid like immunoreactivity (LI).

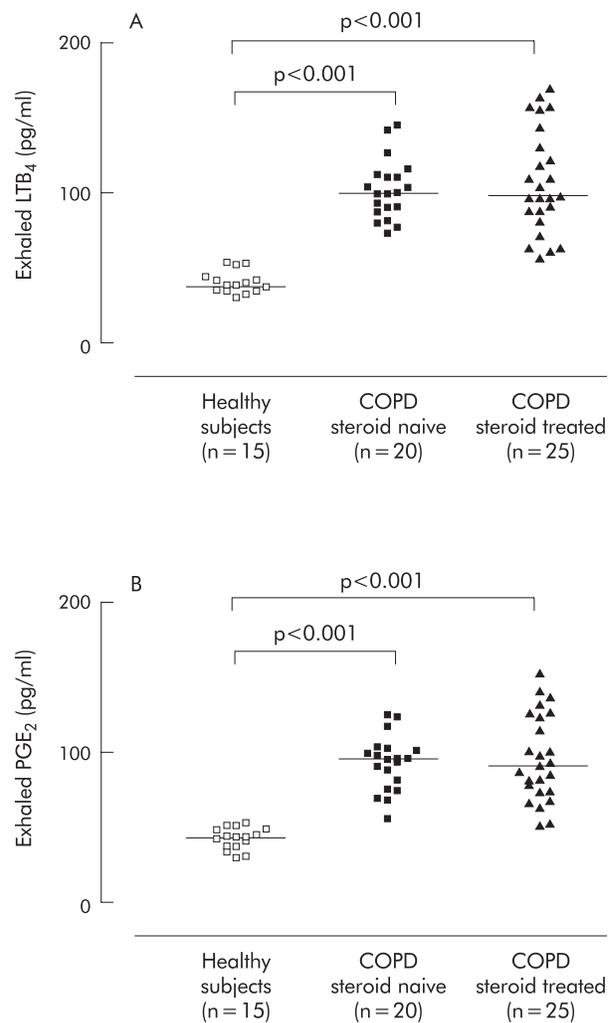
The possible influence of the ventilation rate on exhaled  $LTB_4$ ,  $LTE_4$ -LI, and  $PGE_2$  concentrations was excluded as previously described.<sup>12</sup> Saliva contamination of EBC was excluded by measuring amylase concentrations which were undetectable in all samples tested.

### Statistical analysis

Eicosanoid concentrations in EBC were expressed as median values with the minimum to maximum range. A Kruskal-Wallis test followed by pairwise Mann-Whitney U tests was used to compare groups. Correlation was expressed as Spearman's correlation coefficient. Significance was defined as a p value of <0.05. Within-subject repeatability of exhaled eicosanoid measurements was expressed as intraclass correlation coefficient.<sup>17</sup>

### RESULTS

Exhaled  $LTB_4$  was increased in both steroid naïve COPD patients (100.6 (73.5–145.0) pg/ml;  $p<0.001$ ) and those treated with steroids (99.0 (57.9–170.5) pg/ml;  $p<0.001$ ) compared with age matched controls (38.1 (31.2–53.6) pg/ml; fig 1A).  $PGE_2$  in EBC was also increased in both steroid naïve (98.0 (57.0–128.4) pg/ml;  $p<0.001$ ) and steroid treated patients with COPD (93.6 (52.8–157.0) pg/ml;  $p<0.001$ ) compared with healthy subjects (44.3 (30.2–52.1) pg/ml; fig 1B).

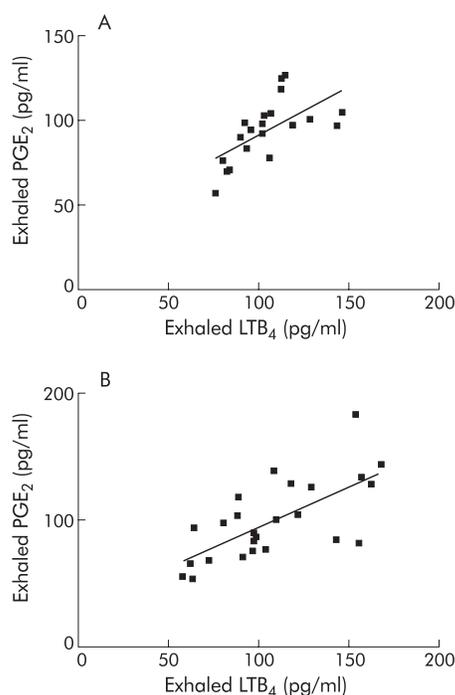


**Figure 1** (A)  $LTB_4$  and (B)  $PGE_2$  concentrations in exhaled breath condensate in healthy subjects ( $\square$ ), steroid naïve patients with COPD ( $\blacksquare$ ), and steroid treated patients with COPD ( $\blacktriangle$ ). Median values are shown by horizontal bars.

There was no difference in exhaled  $LTB_4$  ( $p=0.43$ ) and  $PGE_2$  ( $p=0.59$ ) concentrations between steroid naïve and steroid treated patients with COPD.  $LTB_4$  ( $p=0.86$ ) and  $PGE_2$  concentrations ( $p=0.57$ ) were similar in patients with COPD treated or not treated with theophylline. There was no difference in  $LTB_4$  ( $p=0.89$ ) and  $PGE_2$  concentrations ( $p=0.23$ ) between COPD patients treated or not treated with  $\beta_2$  agonists.

There was no difference ( $p=0.31$ ) in  $LTE_4$ -LI between healthy subjects (15.5 (11.0–27.0) pg/ml), steroid naïve patients with COPD (23.3 (9.1–31.3) pg/ml), and steroid treated patients with COPD (19.5 (9.0–42.0) pg/ml).  $PGF_{2\alpha}$ -LI was measurable in five steroid naïve COPD patients, in six steroid treated patients, and in five healthy subjects. In those subjects in whom  $PGF_{2\alpha}$ -LI was measurable, its concentrations were higher in steroid naïve (15.0 (10.9–19) pg/ml;  $p<0.05$ ) and in steroid treated patients with COPD (14.7 (10.0–21.7) pg/ml;  $p<0.05$ ) than in healthy subjects (8.9 (5.9–10.9) pg/ml).  $PGD_2$ -MOX was measurable in six steroid naïve COPD patients (11.2 (8.7–15) pg/ml), in seven steroid treated COPD patients (11.4 (8.0–14.9) pg/ml), and in six healthy subjects (10.0 (8.0–15) pg/ml). In those subjects in whom  $PGD_2$ -MOX was measurable, there was no difference in the levels of this PG between the three study groups ( $p=0.80$ ).  $TxB_2$ -LI was undetectable in any study subjects.

There was a correlation between  $LTB_4$  and  $PGE_2$  in both steroid naïve ( $r=0.75$ ,  $p<0.001$ ) (fig 2A) and steroid treated



**Figure 2** Correlation between  $LTB_4$  and  $PGE_2$  concentrations in exhaled breath condensate in (A) steroid naïve patients with COPD ( $r=0.75$ ,  $p<0.001$ ,  $n=20$ ) and (B) steroid treated patients with COPD ( $r=0.67$ ,  $p<0.001$ ,  $n=25$ ).

patients with COPD ( $r=0.67$ ,  $p<0.001$ ; fig 2B). The intraclass correlation coefficient was 0.72 for  $LTB_4$ , 0.82 for  $PGE_2$ , 0.68 for  $LTE_4-LI$ , 0.79 for  $PGD_2-MOX$ , and 0.73 for  $PGF_{2\alpha}-LI$ . The intraclass correlation coefficient for  $TxB_2-LI$  was not calculated because this eicosanoid was undetectable in any study subjects.

## DISCUSSION

We have identified the profile of exhaled LTs and PGs in COPD. Patients with COPD had about 2.5-fold higher  $LTB_4$  concentrations in EBC than age matched control subjects, consistent with a primary pathophysiological role for  $LTB_4$  in COPD.<sup>18,19</sup>  $LTB_4$  concentrations in EBC were about 30-fold lower than those reported in sputum.<sup>20</sup> Exhaled  $PGE_2$  was 2.2-fold higher in COPD patients than in healthy subjects. Since  $PGE_2$  may have anti-inflammatory effects in the airways,<sup>21,22</sup> its increase, concomitant with enhanced levels of  $LTB_4$ , a potent pro-inflammatory mediator, may represent a mechanism to counteract lung inflammation in COPD. This hypothesis is further supported by the correlation found between  $LTB_4$  and  $PGE_2$  in both steroid naïve and steroid treated patients with COPD.  $PGF_{2\alpha}-LI$  was detectable in the EBC in a few patients with COPD, and its concentrations were higher than those in healthy subjects. However, because of the small number of patients showing this pattern and the low concentrations detected, increased exhaled  $PGF_{2\alpha}-LI$  in patients with COPD is likely to be of limited biological relevance.

The profile of exhaled eicosanoids in patients with COPD is different from that previously reported in patients with asthma.<sup>12</sup> Exhaled  $PGE_2$  is selectively increased in patients with COPD, whereas  $LTE_4$  is increased in patients with asthma,<sup>12</sup> but not with COPD. Exhaled  $LTB_4$  is increased in both asthmatic and COPD patients, but the increase was more marked in the latter. We have previously shown that exhaled  $TxB_2-LI$  is measurable in about 50% of asthmatic patients,<sup>12</sup> whereas no  $TxB_2-LI$  was detectable in COPD patients in the present study. Identification of selective profiles of exhaled

eicosanoids may help to differentiate between inflammatory lung diseases. The physicochemical properties of eicosanoids in EBC (volatile versus non-volatile) and their partition in EBC (aerosol particles versus water vapour) are largely unknown. The high inter-individual variability in the amount of aerosol particles in EBC indicates the need for a dilution marker.<sup>23</sup> However, the selective increase of structurally related compounds in the single subject ( $LTB_4$  but not  $LTE_4$ , and  $PGE_2$  but not  $PGD_2$ ) is likely to reflect a real increase in the concentrations of these eicosanoids.

The effects of corticosteroids on eicosanoids in COPD in vivo are largely unknown. In our study there was no difference in exhaled LT and PG concentrations between steroid naïve and steroid treated patients with COPD. However, the cross sectional design cannot exclude steroid reduction of  $LTB_4$  or  $PGE_2$  and controlled studies with these drugs are required. Moreover, the effects of potential variables which may influence the effects of exhaled eicosanoids such as the use of previous medication, effects of feeding, effects of exercise, and diurnal variation need to be investigated.

In conclusion, we have shown that  $LTB_4$  and  $PGE_2$  are increased in EBC in patients with stable COPD. The profile of exhaled LTs and PGs in patients with COPD is different from that previously reported in patients with asthma. Identification of selective profiles of eicosanoids in EBC, a completely non-invasive method, may be relevant for the diagnosis and management of COPD.

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## LUNG ALERT

### CT-guided pleural biopsy preferable to traditional Abram's needle in diagnosing malignant pleural disease

▲ Maskell NA, Gleeson FV, Davies RJO. Standard pleural biopsy versus CT-guided cutting-needle biopsy for diagnosis of malignant disease in pleural effusions: a randomised controlled trial. *Lancet* 2003;**361**:1326–30

**M**alignant disease is a leading cause of pleural effusion, with about 40 000 cases occurring annually in the UK and 175 000 in the USA. Pleural fluid cytology establishes a diagnosis in only 60% of these effusions. Pleural biopsy using an Abram's needle, first introduced in 1958, is routinely performed for establishing a diagnosis in the remaining cases. This procedure has a low sensitivity and is associated with numerous complications. Undiagnosed patients may either have to undergo the procedure again or be referred for a thoracoscopic biopsy.

In this single centre, prospective, parallel, randomised study the authors compared CT-guided needle biopsy with standard pleural biopsy using Abram's needle. Fifty patients with cytologically negative unilateral and exudative pleural effusions were randomised to undergo either CT-guided pleural biopsy or Abram's pleural biopsy performed by a single competent operator. Three patients did not undergo the procedure at all and, of the remaining 47, 24 underwent Abram's biopsy while 23 underwent CT-guided biopsy. The results showed that sensitivity for pleural malignancy with the CT-guided procedure was 87% compared with 47% with Abram's needle. Although the specificity and positive predictive value for both CT-guided biopsy and Abram's biopsy were the same at 100% each, the negative predictive value of CT-guided biopsy was superior at 80% compared with 44% with Abram's needle.

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