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Correlation between eicosanoids in bronchoalveolar lavage fluid and in exhaled breath condensate

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Abstract. Exhaled breath condensate (EBC) has been increasingly used as a new and non-invasive method to study airway inflammation. In this study we have compared the concentrations of lipid mediators in EBC with concentrations in bronchoalveolar lavage fluid (BALF).
We included 37 patients undergoing bronchoscopy (12 sarcoidosis, 12 COPD, 6 lung cancer, 5 chronic cough, 1 Wegener's granulomatosis, 1 sclerodermia). Patients were not allowed to have exacerbation or any change in concomitant medication for at least 4 weeks prior to the study. In all patients, EBC was collected immediately prior to the bronchoscopy.
The levels of cys-LTs, LTB_4, 8-isoprostane were significantly higher in BALF compared to EBC ($p < 0.0001$, $p < 0.001$, $p < 0.0001$ for cys-LTs, LTB4, 8-isoprostane respectively). Moreover, there was a strong positive correlation between both leukotriene B_4 and 8-isoprostane in BALF and EBC ($r = 0.53$ and $r = 0.79$, $p < 0.01$, respectively) in patients with sarcoidosis and COPD but there was no correlation between eicosanoids BALF and EBC in patients with chronic cough and lung cancer.
This is the first study to compare EBC and BALF in different lung diseases which demonstrated significant correlations between the levels of eicosanoids in BALF and EBC in patients with COPD and sarcoidosis. EBC may be useful in measuring inflammation in several inflammatory lung diseases.

Keywords: Cysteinyl-leukotrienes, 8-isoprostane, prostaglandin E_2, exhaled breath condensate, bronchoalveolar lavage, bronchoscopy

1. Introduction

Exhaled air contains a large variety of substances, many of which may be markers of local physiological and pathophysiological states in the airways [1]. Exhaled breath condensate (EBC) has been increasingly used as a new and non-invasive method to study airway inflammation [2–5]. EBC has the advantage of being non-invasive and also directly samples mediators from the respiratory tract, thus giving a more direct approach to measuring inflammatory mediators in lung diseases.

EBC contains large number of mediators and their concentrations differ from those found in healthy subjects in several airway diseases, including asthma [2,3,5], chronic obstructive pulmonary disease (COPD) [4], cystic fibrosis (CF) [6,7] and bronchiectasis [8]. Although a research tool at present, EBC may become useful in the diagnosis of patients with various pulmonary diseases in clinical practice. EBC collection is simple to perform, well tolerated by patients while no adverse effects have so far been reported. Moreover, serial measurements can be made with no harmful effects on patients. A 15 minute period of tidal breathing appears to be sufficient to obtain adequate samples. If

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analysis is restricted to a single marker or mediator, only a few minutes of tidal breathing are required for collection of a sample. Sampling can be interrupted at any time.

The volatile and non-volatile substances in human breath can be potentially used in the assessment of airway inflammation based on the assumption that aerosol particles and vapour in exhaled air reflect the composition of the lower airway fluids. It is assumed that airway surface liquid is aerosolized during turbulent airflow and therefore the content of the EBC may reflect the composition of airway surface fluid. However, there is no conclusive data providing clear evidence of the source of particles present in the EBC and only theoretical considerations have been used to conclude that the exhaled breath and exhaled breath condensate mainly represent the biochemical constituents of the lower airways. Therefore, a comparison between EBC and other biological materials from the airways should be performed to validate clinically EBC and evaluate the relationship between markers of inflammation in both EBC and other samples. Bronchoalveolar lavage fluid (BALF) is recognized as a gold standard in analysing local processes in the airways. In this study we have compared the contents of lipid mediators in EBC and BAL fluid in various patients undergoing bronchoscopy in our clinic.

2. Methods

2.1. Study population

We included 37 patients undergoing bronchoscopy for clinical reasons (12 sarcoidosis, 12 COPD, 5 chronic cough, 6 lung cancer, *ca planoepitheliale*, 1 Wegener's granuloma and 1 sclerodermia) (Table 1). We also included 10 healthy age-matched volunteers as a control group but we did not perform bronchoscopy on these subjects due to the difficulty in obtaining consent from the Ethics Committee to use invasive procedures on the healthy population. The bronchoscopy and BAL were performed according to international standards [9]. In all patients EBC was collected immediately prior to the bronchoscopy. All patients were stable with no changes in their symptoms and were medicated for at least 1 month. Patients were allowed to take short-acting inhaled β_2-agonists for symptom control but no steroids were allowed for at least 4 weeks prior to sample collection. All subjects signed a consent form, and the study was approved by the Ethics Committee of the Medical University of Lodz.

2.2. Collection of exhaled breath condensate

The exhaled breath condensate was collected using the condensing device Ecoscreen (Jaeger, Germany). Patients were asked to breathe out spontaneously through a mouthpiece with a saliva trap connected to the tube for 15 min. The respiratory rate ranged between 15–20 breaths/min. Each subject wore a noseclip and rinsed their mouth with distilled water just before and after 7 min of condensing to reduce evaporation of eicosanoids from the saliva and nasal spaces. To determine saliva contamination in all samples (BALF and EBC) amylase was detected (kit from Sigma). Samples were stored at -80° C for not longer than 4 weeks until measurements were taken.

3. Measurement of exhaled eicosanoids

3.1. Leukotrienes

Cysteinyl-leukotrienes (Cys-LTs) were measured by a specific enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbour, MI, USA) as previously described [10]. LTB_4 was measured by an EIA kit (Cayman Chemical). The antiserum used in this assay has 100% cross-reactivity with LTB_4, 39% with 6-trans LTB_4, and $<0.01\%$ each with LTC_4, LTE_4, LTD_4, and LTF_4 and a detection limit of 13 pg/ml for cysLTs and of 4.43 pg/ml for LTB_4 [11].

3.2. 8-isoprostane

8-isoprostane concentration in breath condensate was measured by the EIA kit (Cayman Chemical) as previously described [12]. The minimum detection limit was 5 pg/ml.

3.3. PGE_2

Prostaglandin (PG)E_2 concentration in breath condensate was measured by a specific EIA kit (Cayman Chemical). The antiserum used in this assay has 100% cross-reactivity with PGE_2, 43% with PGE_3, 18.7% with PGE_1, 0.1% each with $PGF_{2\alpha}$, PGA_1, PGA_2 and the detection limit at 4°C is 15 pg/ml.

Table 1
Study population. (mean values ± SD)

Disease	Number of patients	Age (years)	Sex (M:F)	Fev 1 (%)	BALF recovery (%)	EBC volume (ml)
Sarcoidosis	12	39.9 ± 12.3	6:6	83.5 ± 12.3	63.3 ± 8.6	1.82 ± 0.22
COPD	12	54.5 ± 13.4	8:4	67.7 ± 5.9	49.0 ± 7.4	1.48 ± 0.13
Cough	5	38.2 ± 21.7	1:4	88.8 ± 10.5	60 ± 5.0	1.72 ± 0.13
Lung tumor	6	57.8 ± 12.3	3:3	77.2 ± 7.83	51.0 ± 4.9	1.5 ± 0.22
Wegener's granuloma	1	50	0:1	87	60	1.7
Sclerodermia	1	52	0:1	65	65	1.8
Control	10	47.2 ± 17.8	5:5	90.2 ± 9.7	ND	1.88 ± 0.2

Table 2
Eicosanoids (pg/ml) in BALF and EBC in all patients and healthy controls

	Cys-LTs		LTB_4		PGE_2		8-isoprostane	
	BALF	EBC	BALF	EBC	BALF	EBC	BALF	EBC
All patients	34.4 ± 5	20.9 ± 2*	63.9 ± 9	21.9 ± 2**	20.4 ± 3	15.4 ± 1#	63.0 ± 8	17.9 ± 2*
Healthy subjects	N.D.	17.9 ± 6	N.D.	15.5 ± 5	N.D.	17.4 ± 4	N.D.	14.5 ± 5

Abbreviations: Cys-LT = cysteinyl-leukotrienes, LT = leukotriene BALF = bronchoalveolar lavage fluid EBC = exhaled breath condensate N.D. – not done, p values: *$p < 0.0001$ vs BALF, **$p < 0.001$ vs BALF, # $p > 0.05$ vs BALF.

Table 3
Eicosanoids in BALF and EBC in subpopulations of patients according to clinical diagnosis

	CysLTs (pg/ml)		LTB_4 (pg/ml)		PGE_2 (pg/ml)		8-isoprostane (pg/ml)	
	BALF	EBC	BALF	EBC	BALF	EBC	BALF	EBC
Sarcoidosis	31.2 ± 5	22.4 ± 3	38.3 ± 8	25.9 ± 4	11.6 ± 1	8.3 ± 2	80.6 ± 1	21.5 ± 3
COPD	26.6 ± 4	19.0 ± 3	128.4 ± 1	27 ± 2	15.0 ± 2	13.6 ± 2	69.8 ± 1	12.5 ± 2
Lung tumours	98.0 ± 1	67.0 ± 18	65.4 ± 21	26.4 ± 6	67.9 ± 7	29.4 ± 3	19.3 ± 2	11.4 ± 3
Cough	39.5 ± 2	17.2 ± 4	78.6 ± 4	16.3 ± 7	9.3 ± 2	9.9 ± 4	46.4 ± 3	8.4 ± 4
Wegener's granuloma	6.5	6.6	2.0	2.0	23.3	21.2	199.7	45.9
Sclerodermia	6.5	33.2	22.3	22.6	7.5	15.4	23.3	12.5

Abbreviations as for Table 2.

3.4. Statistical analysis

Non-parametric tests were used to compare groups. Levels of measured mediators below detection limit were arbitrarily assumed to be 0.5 of the detection limit. The non-parametric Spearman's rank correlation test was used to assess the relationship between measured parameters. All data is expressed as means ± standard error of the mean and significance was defined as a p value of < 0.05.

4. Results

4.1. The whole study population

Patients' baseline characteristic is presented in the Table 1. Table 2 shows the mean levels of cys-LTs, LTB_4, 8-isoprostane and PGE_2 in BALF and EBC in all patients included to the study. Levels of all mediators except PGE_2 ($p > 0.05$) were significantly higher in BALF compared to EBC ($p < 0.0001$, $p < 0.001$, $p < 0.0001$ for cys-LTs, LTB4, 8-isoprostane respectively). Moreover, there was a strong positive correlation between cys-LTs, LTB_4 and 8-isoprostane in BALF and EBC ($r = 0.72$, $r = 0.53$ and $r = 0.79$, $p < 0.01$, respectively) (Fig. 1).

BALF recovery in the general study population ranged from 45% to 75%. The volume of BALF was significantly lower in lung cancer and COPD patients ($p < 0.01$ vs sarcoidosis) while the EBC volume was the highest in the healthy control and sarcoidosis group and differed significantly from the volume obtained in lung cancer ($p < 0.001$) and COPD patients ($p < 0.001$). (Table 1) Amylase was undetectable in most of samples both EBC and BALF and it was present in 5 of the patients (EBC and BALF) in very low concetrations (4.3 ± 2 units/ml in EBC and 8.5 ± 5 units/ml in BALF).

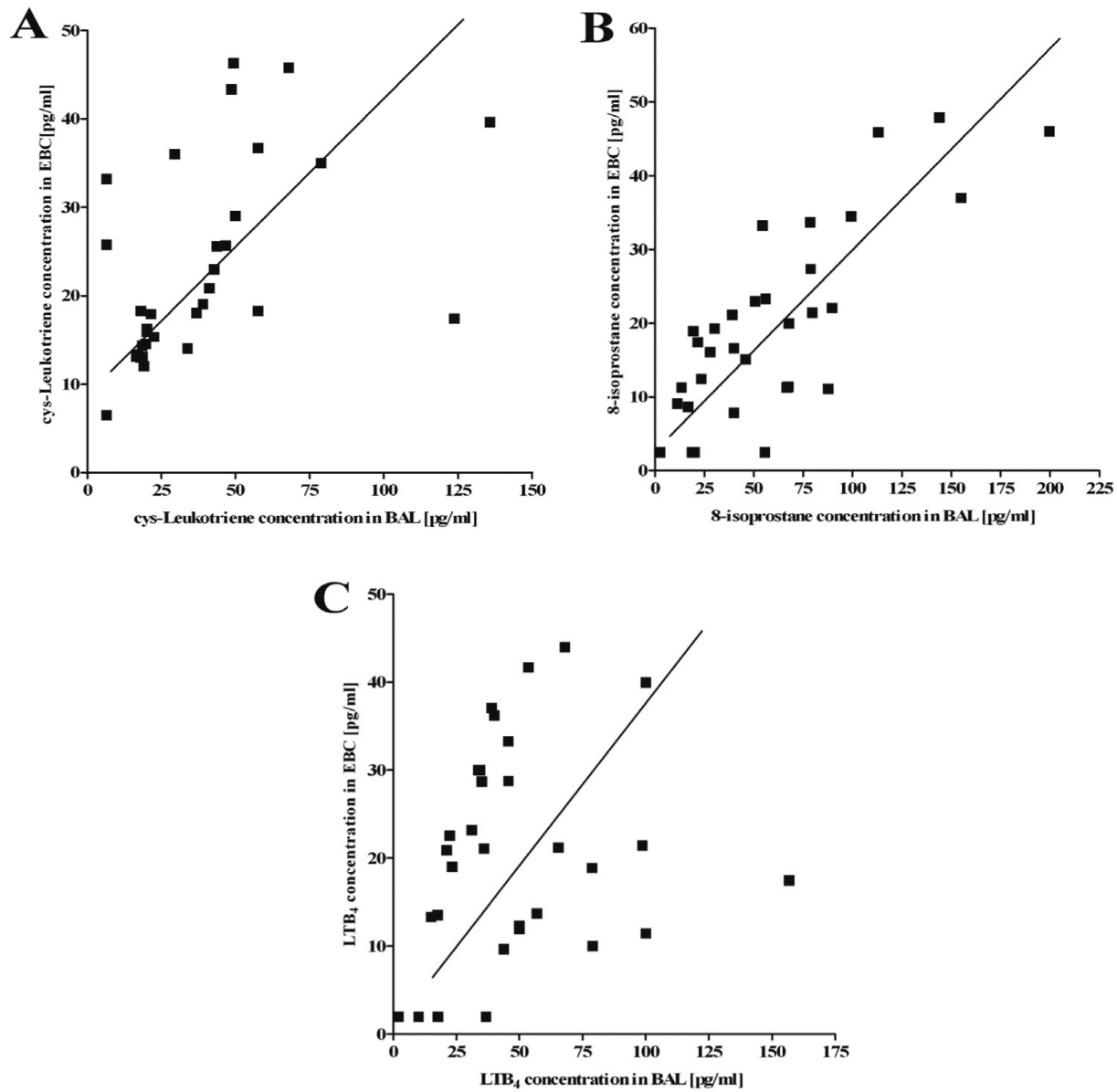

Fig. 1. Correlation between cysteinyl-Leukotrienes (cys-LTs) (panel A), 8-isoprostane (panel B) and leukotriene (LT)B_4 (panel C) in BALF and EBC ($r = 0.72$, $r = 0.79$ and $r = 0.53$, $p < 0.01$, respectively).

4.2. Disease subgroups

Table 3 shows the mean levels of cys-LTs, LTB_4, 8-isoprostane and PGE_2 in BALF and EBC in patients divided into subgroups according to clinical diagnosis.

4.3. Sarcoidosis

The mean concentrations of exhaled eicosanoids from sarcoidosis patients are shown in Table 3. There was a significant difference in lymphocyte counts between sarcoidosis patients and other participants ($23 \pm 6\%$ compared to $8 \pm 2\%$, $p < 0.05$) (Table 4). Levels of all mediators were significantly higher in BALF compared to EBC. As shown in Fig. 2A there was a positive correlation of LTB4 concentration in BALF and EBC ($r = 0.75$, $p < 0.05$). There was also a positive correlation between the levels of 8-isorportane in BALF and EBC ($r = 0.84$, $p < 0.001$) (Fig. 2B). There was no correlation between cell counts in BAL and all

Table 4
The BALF cellular profile in the different disease groups. (mean values ± SD)

	Sarcoidosis	Lung tumor	COPD	Cough	Wegener's granuloma	sclerodermia
macrophages	72.2 ± 21.1	86.8 ± 5.6	86.3 ± 6.3	87.6 ± 6.8	70	88
lymphocytes	23.2 ± 21.3	8.8 ± 2.6	8.25 ± 5.3	8.8 ± 6.9	5	7
neutrophils	1.3 ± 1.2	2.6 ± 3.6	2 ± 1.7	1.4 ± 1.5	21	1
monocytes	1 ± 0.9	0.8 ± 1.3	1.3 ± 1.0	0.6 ± 0.9	0	0
eosinophils	1.25 ± 1.3	0.2 ± 0.4	1 ± 0.9	0.8 ± 0.8	0	4
basophils	0.5 ± 1	0.4 ± 0.5	0.75 ± 0.7	0.6 ± 0.5	0	0
epithelium	0.25 ± 0.5	0	0.6 ± 0.6	0.2 ± 0.4	4	0
vitality %	98.3 ± 0.9	98 ± 1	98 ± 1.2	98.6 ± 0.9	99.0	100

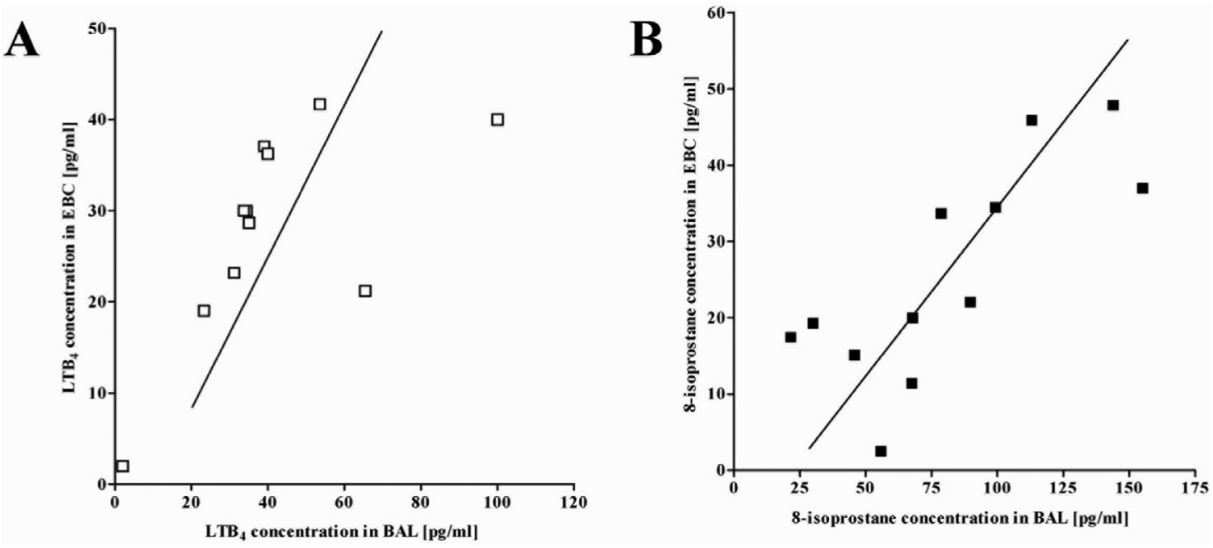

Fig. 2. Positive correlation of LTB_4 concentration in BALF and EBC ($r = 0.75$, $p < 0.05$) (A). Positive correlation between levels of 8-isoprostane in BALF and EBC in patients with sarcoidosis ($r = 0.84$, $p < 0.01$) (B).

mediators measured in both BALF and EBC. Furthermore, patients with active sarcoidosis, as measured by BAL lymphocytes $> 18\%$ had significantly higher levels of 8 isoprostanes in both BALF and EBC compared to patients with nonactive disease (BALF 95.5 ± 24.7 and 70 ± 11.1, $p < 0.0001$, EBC 27.2 ± 6.7 and 24.4 ± 5.5, $p < 0.001$ for patients with active and nonactive sarcoidosis respectively). Finally, there was no correlation between cell counts in BAL and mediator concetrations measured in BALF and EBC.

4.4. COPD

The mean concentrations of exhaled eicosanoids from COPD patients are shown in Table 3. As shown in Fig. 3A, there was a positive correlation of 8-isoprostane concentration in BALF and EBC ($r = 0.67$, $p < 0.05$). There was also a positive correlation between the levels of LTB_4 in BALF and EBC in patients with COPD ($r = 0.8$, $p < 0.01$) (Fig. 3B). There was no correlation between cell counts in BAL and all mediators measured in both BALF and EBC.

4.5. Lung tumours

The mean concentrations of exhaled eicosanoids from patients with lung tumours are shown in Table 3. There were high concentrations of cys-LTs and surprisingly PGE_2 in EBC compared to healthy controls (cys-LTs 67.0 ± 18 compared to 17.9 ± 6 pg/ml, $p < 0.05$ and 29.4 ± 3 compared to 17.4 ± 4 pg/ml, $p < 0.05$, respectively).

In general, there was no correlation between the concentrations of eicosanoids in BALF and EBC and there was no correlation between cell counts in BAL and all mediator concetrations measured in BALF and EBC.

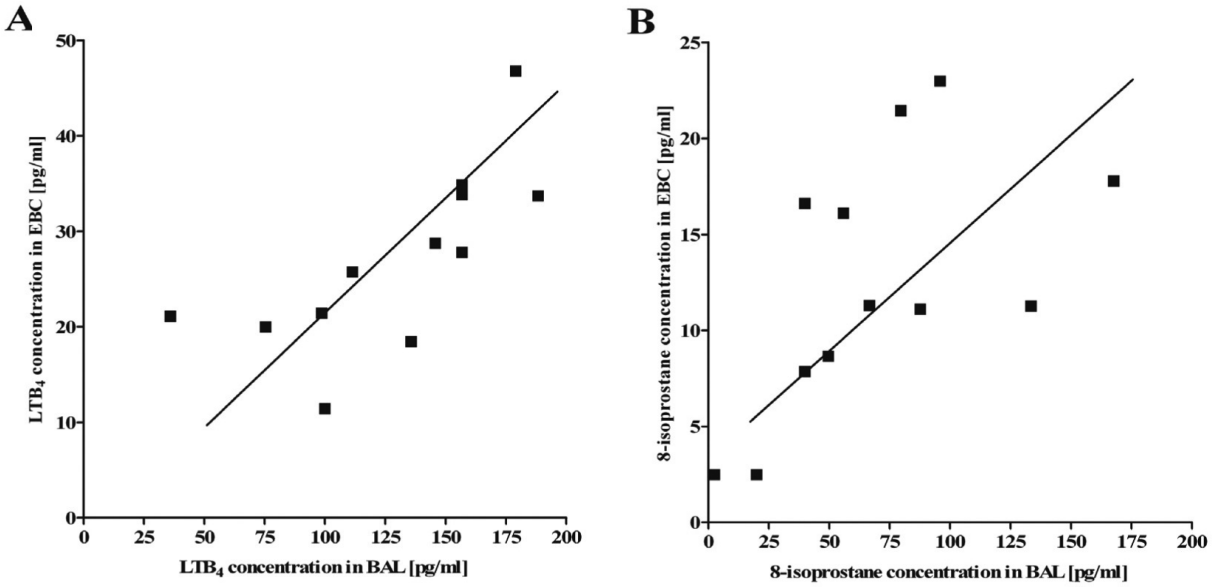

Fig. 3. Positive correlation between levels of LTB_4 in BALF and EBC in patients with COPD ($r = 0.8$, $p < 0.01$) (A). Positive correlation of 8-isoprostane concentration in BALF and EBC ($r = 0.67$, $p < 0.05$) (B).

4.6. Idiopathic cough

There were 5 patients undergoing bronchoscopy due to a cough of unknown origin. In this group we found significantly higher levels of LTB_4 and 8-isoprostane in EBC compared to healthy controls (see Table 3 and 2). There was no correlation between eicosanoids BALF and EBC in this group and there was no correlation between cell counts in BAL and mediator concetrations measured in BALF and EBC.

5. Discussion

It has been proposed that the composition of EBC reflects the biochemical contents of the lower airways. Therefore, a comparison between EBC and other biological materials from the airways seems to be justified to validate EBC and evaluate the relation between markers of inflammation in both EBC and other samples. As BALF is recognised as one of the gold standards in analysing local inflammatory processes in the airways (8) we compared the content of inflammatory markers in EBC and BAL fluid in various patients undergoing bronchoscopy for several lung diseases.

In this study we have shown that concentrations of cys-LTs, LTB_4 and 8-isoprostane were significantly higher in BALF compared to EBC. In general they were also higher in EBC in this study population compared to healthy controls. Moreover, there was a positive correlation between the levels of LTB_4 and 8-isoprostane in BALF and EBC measured in patients with sarcoidosis and COPD, but there were no correlations between the concentrations of eicosanoids in BALF and EBC in patients with chronic cough and lung tumors. We suppose that the lack of correlations may result from the small number of patients in these subgroups.

There was no correlation between cell counts in BAL and mediator concetrations measured in BALF and EBC. However, sarcoidosis patients with active disease as measured by BAL lymphocytes of $>18\%$ (lymphocyte alveolitis) had significantly higher levels of 8-isoprostanes in both BALF and EBC compared to nonactive sarcoid patients. This is consistent with the observation of Psathakis et al. [13] and Piotrowski [14] who found that 8-isoprostane is increased in EBC from patients with active sarcoidosis and correlates with serum angiotensin-converting enzyme (sACE) [13], thus implicating the measurement of 8-isoprostane as an index of disease activity.

This study shows a positive correlation between well known lipid mediators such as LTB_4 and 8-isoprostane in EBC and BALF. BALF directly samples mediators from the respiratory tract, thus giving a direct approach to the insight of inflammatory mediators in the airways. Moreover, the cellular content is another source of information and also a source of the mediators. The similarity between BALF and EBC allows us to draw

a conclusion that the mediators we measured in both materials come from the same source which might be phagocytes present in airway lumen, epithelial cells and pneumocytes [15]. The higher concentrations of lipid mediators in BALF compared to EBC might be derived from cells present in the fluid and to an unknown extent from mediators that are washed out from the epithelial lining fluid.

In EBC there are lower levels of all mediators which are likely to be due to the dilution of droplets generated in the respiratory tree by water vapor. The dilution of droplets containing inflammatory mediators in this case is inevitable. However, changes in their concentrations in condensate vary by a factor of less than 5, depending on variations in the dilution, which is not consistent with the data from Effros and coworkers [16] who suggested a dilution factor in condensate droplets of 100 or more. The correlation between levels of mediators present in both BALF and EBC show that EBC can be a valuable technique to assess pathological events in the airways.

Both LTB_4 and 8-isoprostane concentrations in EBC and BALF were correlated in sarcoidosis and in COPD patients. Exhaled LTB_4 has been reported to be increased in COPD patients [17,18]. COPD seems to be strongly neutrophil-dependent and it is no surprise to find increased LTB_4 in COPD as this mediator is a potent neutrophil chemoatractant in the airways. However, this is the first study to demonstrate a correlation between mediator concentrations in BALF and EBC. 8-isoprostane was recently reported to be increased in EBC in patients with sarcoidosis [13,18], consistent with the increased concentrations previously demonstrated in BALF [19]. This suggests that oxidative stress is present in the airways in sarcoidosis. This is the first study in which EBC and BALF constituents have been performed at the same time. Surprisingly, we found increased LTB_4 in both BALF and EBC in patients with sarcoidosis.

It seems that LTB_4 may play a pathological role in sarcoidosis through the recruitment of neutrophils. It has been suggested that cyclooxygenase and lipooxygenase pathway metabolites of arachidonic acid modulate the evolution of the granulomatous inflammatory response in the lung. Moreover, stimulated alveolar macrophages from patients with active sarcoidosis release higher levels of LTB_4 compared to healthy controls [20]. This could contribute to the locally inflammatory response. Interestingly, in patients with lung tumours, we found increased levels of cys-LTs and PGE_2 in BALF and EBC. They play different roles in biological systems – cys-LTs are potent bronchoconstrictor and pro-inflammatory mediators [21], while PGE_2 has anti-inflammatory effects [22]. The role of lipid mediators in cancer is unknown.

An important question is whether measuring inflammatory markers in EBC is as good as the measurement of these compounds in the much more invasive BALF. EBC collection is simple and non-invasive whereas BAL is invasive and this means that repeated measurements are not possible and that it cannot be routinely used in patients with severe disease or in children. Taking the above into consideration EBC would be a useful method especially in monitoring disease activity, course, and response to treatment, as repeated sampling is possible. As mentioned by Psathakis [13] EBC represents the whole lung sample when BAL is locally-limited to a portion of the lung and may not give an insight to all the airways. Further studies are needed to compare EBC and BAL methods and their potential use in diagnosis, monitoring diseases and response to therapy.

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