



Leukotrienes and 8-isoprostane in exhaled breath condensate in bronchoprovocation tests with occupational allergens

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Abstract

Exhaled breath condensate (EBC) contains many substances, which could help in diagnosis of occupational asthma. The aim of the study is to monitor leukotrienes (LT) and 8-isoprostane from EBC in bronchoprovocation tests with allergens in 47 patients with suspected occupational asthma. Forty-one patients were tested negative. In negative bronchoprovocation tests, no significant differences ($P < 0.05$) were seen between the five measurements during and after the test. In control measurements (without provocation), significant differences were found among four measurements done within 24 h for 8-isoprostane ($P = 0.0138$). The relationship between the log transformed ratios of the EBC parameters and FEV₁ was never significant at the 5% level in control measurements, while in negative tests, statistical significance was recorded for LTB₄ ($P = 0.0299$) before and 5 h after the test. Six of 47 patients were tested positive. Such a small number of patients did not allow proper statistical analysis and therefore, the results are described separately for each patient.

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1. Introduction

The proper diagnosis of occupational asthma is important for the prognosis of the disease. This diagnosis can be based on some tests. Controlled exposure to suspected occupational allergens in the laboratory is considered to be the gold standard [1]. Occupational allergens are either applied with a special bronchoprovocation device, or the patients are exposed to allergens in the challenge chamber under conditions reproducing the workplace exposure. An integral part of the specific bronchoprovocation tests is the monitoring

of subjective complaints, objective findings and the repeated lung function measurement over 24 h. Exposure to allergens can be performed also directly in the workplace. In most countries specific bronchoprovocation tests are not commonly used because of their exacting scheme and the need for hospitalization of the patients. They are substituted by serial measurements of peak expiratory flow (PEF) at rest and in the workplace, which requires good co-operation from the patients [2–4].

Our department has been performing bronchoprovocation tests both in the laboratory and in the workplace for many years. Nevertheless, it is not always possible to make an unequivocal diagnosis of occupational asthma. It may pose difficulty especially in the case of a borderline decrease in ventilatory parameters, or, on

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the other hand, in patients with advanced or persisting obstructive ventilatory defect, in whom the specific bronchoprovocation tests cannot be performed. If the specific test is not feasible, other tests (and their combination) like non-specific bronchoprovocation tests, skin prick tests, serum-specific immunoglobulin E (IgE) can be helpful in the determination of final diagnosis (each with various specificity and sensitivity compared to the specific bronchoprovocation test) [5].

Patients on permanent antiasthmatic treatment whose reaction to allergens is partially mitigated by the treatment are frequently referred to our department for bronchoprovocation testing. Therefore, a need arises to look for other markers (in addition to the ventilatory parameters), which would enable the verification of the reaction to the occupational allergen. Induced sputum analysis is one of the procedures previously reported to be helpful in occupational asthma diagnosis. However, it has the disadvantage that, despite the induction of sputum by hypertonic saline, some people do not produce a satisfactory sample of sputum for analysis. Multiple studies have found elevation of eosinophils in sputum after a positive bronchoprovocation test [6]. Exhaled nitric oxide (NO) could be another helpful marker in occupational asthma diagnosis. A definite advantage is that its collection is non-invasive. The sticking point is the lack of unambiguous data after the allergen testing [6].

Exhaled breath condensate (EBC) contains many substances reflecting changes in the airways and it offers further possibilities for the diagnosis and monitoring of several respiratory diseases. Recently, utilization of EBC has been discussed extensively.

Cysteinyl leukotrienes are bioactive lipids. Cysteinyl leukotrienes C₄, D₄, and E₄ are formed by the biotransformation of arachidonic acid. Assessment of in vivo production of cysteinyl leukotrienes was based, until recently, almost exclusively on the urinary LTE₄ concentration. However, this concentration reflects the total production of leukotrienes in the body and not their separate production in the airways. Collection of the EBC enables us to study the concentration of leukotrienes directly in the airways and to detect not only the final LTE₄, but also the main contractile leukotrienes LTC₄ and LTD₄ [7].

The other parameters of interest for the monitoring during the bronchoprovocation are leukotriene B₄ and 8-isoprostane, known to be elevated in asthmatics [8–12]. Leukotriene B₄, a potent chemoattractant of neutrophils, has a weak effect on smooth muscle cells but may contribute to airway narrowing by producing local swelling and increasing mucus secretion [13]. 8-isoprostane, an F₂ isoprostane, formed nonenzymatically by oxidation of arachidonic acid, is a stable marker of oxidative stress. The 8-isoprostane level was elevated

in EBC of asthmatic patients and reportedly correlates with disease severity [10–12].

The studies using analysis of LTE₄ as the final metabolite of cysteinyl leukotrienes in urine after bronchoprovocation tests with allergens have observed elevated LTE₄ concentrations during early asthmatic reaction (EAR) and/or late asthmatic reaction (LAR). Kumlin et al. [14] have reported the inhalation of specific allergens in atopic asthmatics to be followed by increased levels of LTE₄ and 11-dehydro-TXB₂ in urine. Taylor et al. [15] and Smith et al. [16] have found elevated LTE₄ in urine, collected for 3 h after antigen inhalation. Manning et al. [17] studied urinary LTE₄ during early and late asthmatic responses. The degree of maximal bronchoconstriction during the early response correlated with the LTE₄ levels. No significant increase in urinary LTE₄ levels occurred during the late response. On the other hand, O'Sullivan et al. [18] have reported increased urinary LTE₄ both in the early and late phase airway response. In aspirin-sensitive asthmatics, a significant increase in LTE₄ urinary concentration followed bronchial provocation with aspirin [14,19,20]. These findings support our hypothesis that leukotrienes in EBC could be elevated after a positive bronchoprovocation test with the occupational allergen.

The aim of our study is to monitor changes in EBC concentrations of leukotrienes (LT) and 8-isoprostane prior to and after bronchoprovocation tests in patients with suspected occupational asthma and/or rhinitis.

2. Patients and methods

2.1. Study subjects

Forty-seven subjects enrolled in the study were patients of the Department of Occupational Medicine, Prague, Czech Republic, who had presented with suspected occupational asthma and/or rhinitis. The patients had medical history of bronchial asthma, rhinitis or cough and a workplace allergen was considered as a probable cause of their allergic symptoms. The aim of our work is to perform bronchoprovocation tests with these suspected occupational allergens.

All patients underwent the following clinical and laboratory examinations: eosinophils, total IgE and eosinophil cationic protein (ECP) from blood, skin prick tests, control 24-h measurement at rest without provocation (to make sure of the stability of spirometry parameters), non-specific bronchoprovocation test with histamine, and specific bronchoprovocation tests with suspected allergens. Three patients were tested while on permanent treatment with inhaled corticosteroids. Characteristics of the study subjects are summarized in Table 1.

2.2. Methods

2.2.1. Lung function

Spirometry was performed using both the MasterLab and MasterScreen units (Jaeger, Germany) in line with the European Respiratory Society recommendations [21].

Control measurements without provocation were done in all patients. Spirometry was performed four times within 24 h and was timed to match the measurements during the bronchoprovocation test as follows: time 1 (between 7 a.m. and 10:15 a.m.), time 2 (between 10:30 a.m. and 2 p.m. on the same day), time 3 (between 1:30 p.m. and 5:30 p.m. on the same day), and time 4 (between 7:00 a.m. and 10:15 a.m. on the next day).

The non-specific bronchoprovocation test was performed with histamine using the dosimeter method (Asthma Provocation System, Jaeger, Germany). Histamine (histamine dihydrochloride) was inhaled at concentrations of 1, 5, and 10 (15) mg/ml. A decrease in forced expiratory volume measured in 1 s (FEV₁) exceeding 20% was considered as a positive result [22,23].

Specific bronchoprovocation tests were done with suspected allergens from the patient's workplace. Exposure to allergens lasted 30 min and spirometry followed immediately after the test; 2, 5, and 24 h after the test; or any time when the patient reported some

respiratory symptoms. A positive result was based on the following criteria: (1) the decrease in FEV₁ exceeded 20% or (2) the decrease in mean expiratory flow at a specified (25%, 50%, 75%) lung volume (MEF_{25%}FVC, MEF_{50%}FVC, MEF_{75%}FVC) exceeded 30% while the decrease in FEV₁ exceeded 15% or (3) the increase in total airways resistance (R_{tot}) exceeded 70% of the pre-test values while the decrease in FEV₁ exceeded 15%.

2.2.2. Skin prick tests

Skin prick tests were done with the extracts of common environmental respiratory allergens (dust, feathers, mites, grass pollen in spring and autumn mixtures, moulds, and upper airway bacteria from Sevapharma, Stallergenes). A positive reaction was defined as a weal with a diameter of not less than 3 mm.

2.2.3. EBC collection

EBC samples were collected using a commercially manufactured condenser EcoScreen (Jaeger, Höchberg, Germany) into standard plastic tubes. The condenser is a system consisting of a mouthpiece with a one-way valve and a collecting system. The study subjects breathed through the mouthpiece connected to the valve block in which inspiratory and expiratory airflows are separated. The valve block is connected to a cooling system inserted into a cooling cuff maintained at a cold temperature by a refrigerator (−10 °C, as required by the manufacturer) [24]. Patients were instructed to breathe tidally for approximately 20 min while wearing a nose-clip. The volume of the samples was about 2 ml. Samples were frozen immediately after collection (−80 °C).

Breath condensate was collected during the control measurements without provocation four times within 24 h, with the collection timed to match the measurements during the test, and five times in the specific bronchoprovocation test as indicated for spirometry. Spirometry measurement followed immediately after each EBC collection.

Table 1
Characteristics of examined patients

<i>n</i> = 47	
Age (years) ± SD	44.0 ± 10.2
Sex (men/women)	18/29
Smoking (non-smoker/ex-smoker/smoker)	22/16/9
ECP (ng/ml) ± SD	42.0 ± 34.5
Blood eosinophils (%) ± SD	4.1 ± 4.1
Blood eosinophils (absolute count) ± SD	0.28 ± 0.42
Total IgE (positive/negative)	16/31
Prick tests (positive/negative/not done—not applicable)	20/23/4
Histamine bronchoprovocation test (positive/negative)	29/18

Notes: Data are either mean ± standard deviation or number of persons.

Table 2
Mean values and standard deviations of FEV₁ and exhaled breath condensate parameters from the control measurement without provocation

Control measurement without provocation	Time point 1	Time point 2	Time point 3	Time point 4
FEV ₁ (SD) (%predicted)	98.0 (16.5)	97.5 (16.2)	97.1 (15.2)	97.4 (14.0)
LTB ₄ (SD) (pg/ml)	39.2 (15.0)	35.2 (14.4)	39.7 (13.7)	37.6 (11.0)
LTC ₄ (SD) (pg/ml)	56.7 (21.9)	64.7 (28.4)	65.8 (25.1)	64.3 (25.1)
LTD ₄ (SD) (pg/ml)	24.9 (13.6)	23.2 (11.3)	24.5 (9.4)	22.5 (10.5)
LTE ₄ (SD) (pg/ml)	41.8 (19.9)	45.8 (23.4)	50.3 (21.8)	45.2 (22.9)
8-ISO (SD) (pg/ml)	69.4 (27.2)	61.7 (24.6)	65.2 (26.7)	73.8 (27.4)

Notes: LT: leukotriene; 8-ISO: 8-isoprostane; FEV₁: forced expiratory volume measured in 1 s; SD: standard deviation. Intervals of EBC collection: time 1—between 7 a.m. and 10:15 a.m.; time 2—between 10:30 a.m. and 2 p.m.; time 3—between 1:30 p.m. and 5:30 p.m.; time 4—between 7 a.m. and 10:15 a.m. on the next day.

2.2.4. EBC analysis

Immunoseparation with commercial cysteinyl leukotriene affinity sorbent (Cayman Chemicals, USA) and 8-isoprostane affinity sorbent (Cayman Chemicals, USA) was conducted as follows: 1 ml of EBC, 50 μ l of each of the immunoaffinity sorbents, and 100 pg of each of the deuterium-labeled standards ([6,7,14,15, ^{2} H] leukotriene B₄, [17,18,19,20, ^{2} H] 8-isoprostane (Cayman Chemical, USA), [6,7,14, ^{2} H] leukotriene E₄ (Biomol, USA)) were mixed, the suspension was shaken for 120 min at 20 °C and subjected to centrifugation, followed by sorbent separation and washing with water (2 $\times$ 1 ml). After that, methanol (2 $\times$ 0.5 ml) was added for substrate elution from the antibody–substrate complex; the suspension was shaken for 5 min and then centrifuged with subsequent supernatant separation. The solvent was removed from the supernatant (stripping with nitrogen) and 50 μ l of a mobile phase was added. After these steps samples were ready for LC/MS analysis.

2.2.5. LC/MS method

EBC concentrations of each of the analytes (markers) were determined by the LC/MS method (Varian 1200L, Varian, USA; column Hypercarb Thermo

100 $\times$ 2.1 mm $\times$ 5 μ m, Mobile phase: Acetonitrile: Water:TEA 70:30:pH = 11, flow = 200 μ l/min; ESI negative, MRM mode: LTB₄ (Q1 parent ion 335.1 $\rightarrow$ Q3 daughter ion 194.1; CID energy 18 V), ^{2} H LTB₄ (339.1 $\rightarrow$ 198.1; 18 V), LTC₄ (624.1 $\rightarrow$ 271.1; CID 27 V), LTD₄ (495.2 $\rightarrow$ 176.1; 21 V), LTE₄ (438.2 $\rightarrow$ 333.1; 19 V), ^{2} H LTE₄ (441.2 $\rightarrow$ 336.1; 20 V), 8-isoprostane (353.1 $\rightarrow$ 192.9; 29 V), ^{2} H 8-isoprostane (353.1 $\rightarrow$ 192.9; 28 V), capillary voltage -70 V, CID gas (Ar) pressure 0.2 Pa.

The limit of detection (LOD), the limit of quantification (LOQ), and recovery for particular analytes were as follows: LTB₄—LOD = 1 pg/ml, LOQ = 4 pg/ml, recovery = 46%; LTC₄—LOD = 2 pg/ml, LOQ = 16 pg/ml, recovery = 55%; LTD₄—LOD = 1 pg/ml, LOQ = 6 pg/ml, recovery = 61%; LTE₄—LOD = 1 pg/ml, LOQ = 5 pg/ml, recovery = 81%; 8-isoprostane—LOD = 1 pg/ml, LOQ = 5 pg/ml, recovery = 84%. The imprecision was statistically evaluated as lower than 8%.

2.2.6. Contamination of saliva

The contamination of saliva in EBC samples was determined by the colorimetric detection of α -amylase (α -amylase-liquid BIO-LA-TEST kit, Pliva-Lachema,

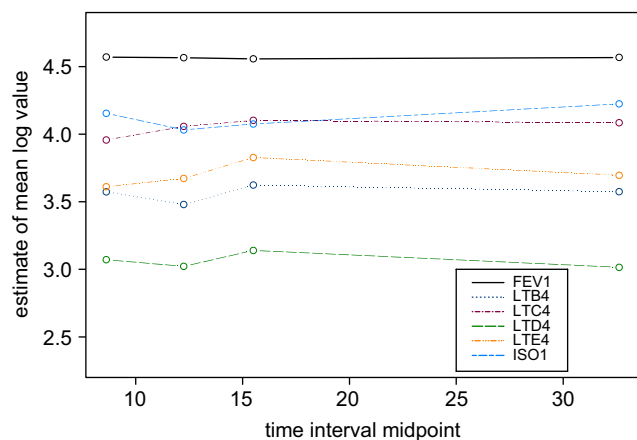


Fig. 1. Comparison of estimates of the expected values of logs obtained from the LME model for separate parameters in measurements at rest (without provocation). Notes: LT: leukotriene; ISO 1: 8-isoprostane; FEV1: forced expiratory volume measured in 1 s.

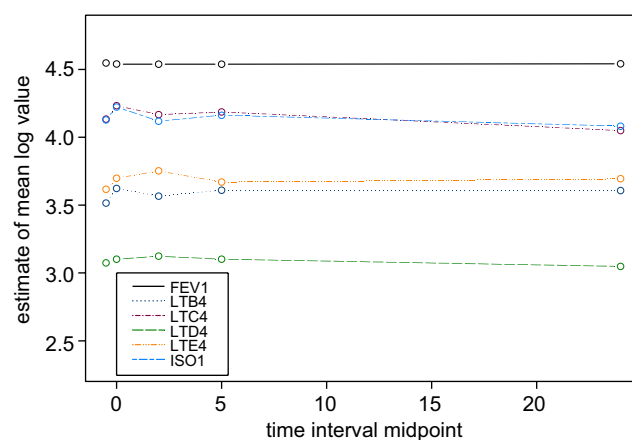


Fig. 2. Comparison of estimates of the expected values of logs obtained from the LME model for separate parameters in negative bronchoprovocation tests. Notes: LT: leukotriene; ISO 1: 8-isoprostane; FEV1: forced expiratory volume measured in 1 s.

Table 3

Mean values and standard deviations of FEV₁ and exhaled breath condensate parameters measured in negative bronchoprovocation tests

Negative tests	Baseline	After test	2 h after test	5 h after test	24 h after test
FEV ₁ (SD) (%predicted)	95.3 (19.5)	94.7 (20.0)	94.5 (19.2)	94.6 (19.4)	94.7 (17.2)
LTB ₄ (SD) (pg/ml)	38.1 (19.2)	42.3 (21.0)	40.4 (19.2)	39.7 (16.1)	40.3 (17.3)
LTC ₄ (SD) (pg/ml)	74.4 (51.5)	76.0 (48.7)	72.3 (37.7)	74.4 (38.6)	63.3 (25.0)
LTD ₄ (SD) (pg/ml)	24.3 (11.8)	24.4 (10.5)	24.4 (9.2)	25.3 (15.4)	23.2 (10.5)
LTE ₄ (SD) (pg/ml)	42.8 (22.4)	48.2 (30.4)	50.3 (27.0)	45.5 (25.2)	45.3 (22.0)
8-ISO (SD) (pg/ml)	65.5 (24.4)	72.3 (28.3)	67.8 (34.0)	70.2 (30.6)	64.1 (24.0)

Notes: LT: leukotriene; 8-ISO: 8-isoprostane; FEV₁: forced expiratory volume measured in 1 s; SD: standard deviation.

Czech Republic). In most of the samples, α -amylase was not detected (in the rest of the samples α -amylase activity/nkat/ μ l was below 0.2).

2.2.7. Statistical analysis

The concentration data (leukotrienes and isoprostane) are skewed to the right. Therefore, logarithmic transformation of the concentrations before the (normal) analyses was used. The data generally conform with lognormality (i.e. with normality after taking log transformation). Regressions of leukotriene (or isoprostane) values (as “potential markers”) against FEV₁ were done. A sort of “individual standardization” was used before relating marker concentration to FEV₁. The ratio of the current value to the initial value (both for a marker and for FEV₁) was taken. The ratios were then logarithmically transformed and related through a regression model formally stated below. This statistical

model was used as it involves the transformed relative value of an EBC parameter concentration and the transformed relative FEV₁ value as “a gold standard”. The formal model for a particular marker concentration (in control measurements at rest for $i = 2, 3, 4$ or in negative tests for $i = 2, 3, 4, 5$) is

$$\log\left(\frac{conc_{it}}{conc_{i1}}\right) = a + b \cdot \log\left(\frac{FEV1_{it}}{FEV1_{i1}}\right) + \varepsilon_{it} \quad (1)$$

where $conc_{it}$ is the marker concentration (e.g. of LTC₄) for patient i at time point t (we had four time points in control measurements at rest or five time points for the negative bronchoprovocation test), a and b are “calibration” coefficients, and ε_{it} is a random error assumed to be normally independently distributed with zero mean.

ANOVA-like analyses were computed for logarithmically transformed measurements too, with the time

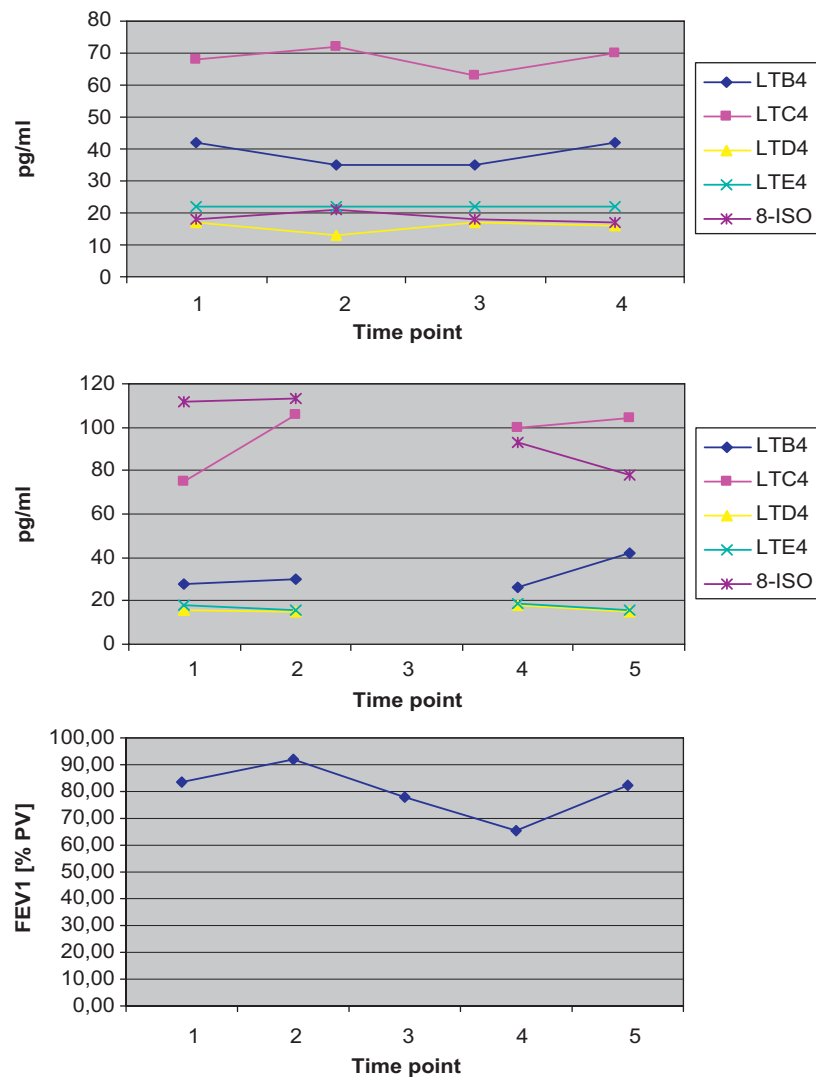


Fig. 3. Patient 1—concentrations of leukotrienes and 8-isoprostane during the monitoring at rest and positive bronchoprovocation test (late asthmatic reaction at time point 4).

factor in the LME (linear mixed effects) model with random (or individual-specific) intercepts. The LME model is described as

$$\log(\text{conc}_{it}) = a_i + \eta_i + b_i \log(\text{FEV}_1) + \varepsilon_{it} \quad (2)$$

where conc_{it} is the marker concentration (e.g. of LTC_4) for patient i at time point t , a_i, b_i are unknown parameters, η_i are individual-specific intercept departures (random effects and they are assumed to follow independently normal distribution with zero mean and standard deviation—SD σ_η), ε_{it} is a random error assumed to be normally independently (and independently from η_i 's) distributed with zero mean and SD σ .

The study was carried out according to the Helsinki Declaration. The Ethics Committee of the 1st Faculty of Medicine, Charles University, approved all examinations and tests, and all of the study subjects gave their written informed consent for all tests and examinations.

3. Results

3.1. Negative tested patients

As many as 41 of 47 patients were tested negative. In these patients, the control measurements at rest (without provocation) were done, the bronchoprovocation test was negative (based on the ventilatory parameters).

3.2. Control measurements at rest in negative tested patients

The mean values of FEV_1 , LTB_4 , LTC_4 , LTD_4 , LTE_4 , and 8-isoprostane with SD from the four measurements within 24 h, timed to match the measurements during the following bronchoprovocation test, are given in Table 2.

ANOVA-like analyses were computed for logarithmically transformed measurements, with the time factor (four repeated measurements for all markers and FEV_1)

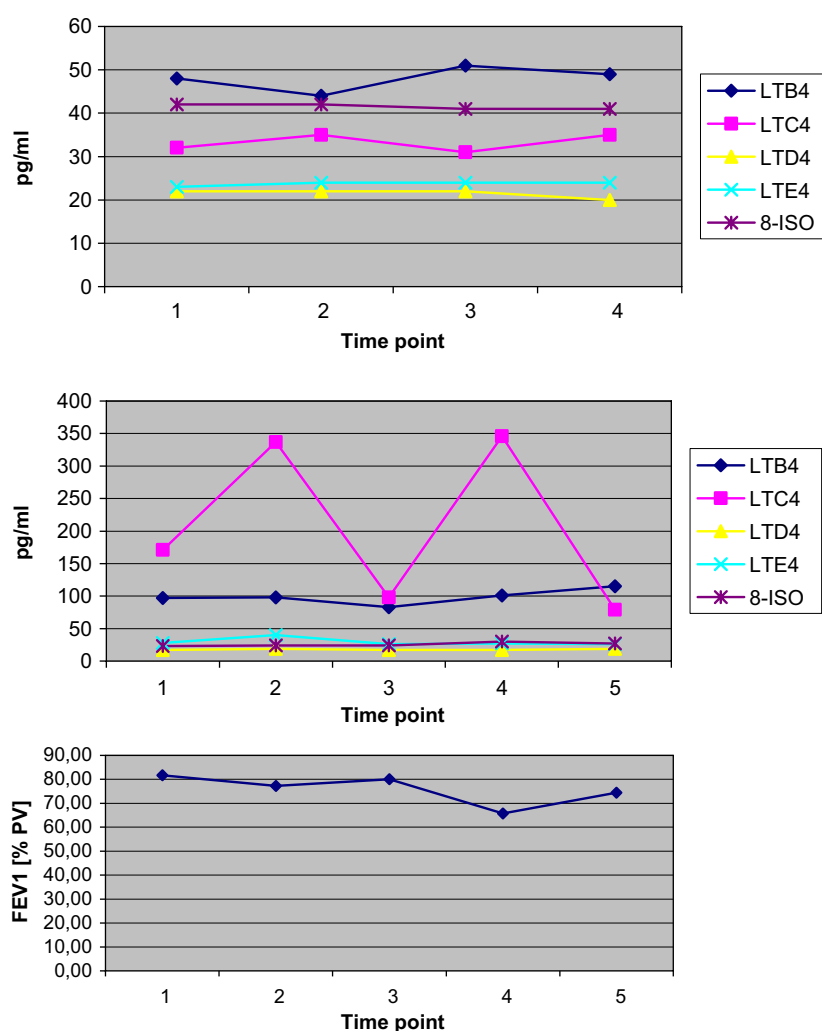


Fig. 4. Patient 2—concentrations of leukotrienes and 8-isoprostane during the monitoring at rest and positive bronchoprovocation test (late asthmatic reaction at time point 4).

in the LME model with random (or individual-specific) intercepts. *P*-values of the tests for the null hypothesis that there are no changes over the four measurements (spanning from the morning of the first day to the next morning) were the following: FEV₁ 0.634, LTB₄ 0.1185, LTC₄ 0.0889, LTD₄ 0.4027, LTE₄ 0.0504, and 8-isoprostane 0.0138. Therefore, we can see significant changes for 8-isoprostane, while changes in LTE₄ and LTC₄ are rather close to the borderline.

Fig. 1 compares estimates of the expected values of logs obtained from the LME model for different parameters (for each parameter separately). In contrast to most of the markers, FEV₁ does not seem to change over time. The differences are significant only for 8-isoprostane (as it was said earlier).

The relationship between the log transformed ratio of each EBC parameter and FEV₁ was tested too. The

P-value for the test of null hypothesis about $b = 0$, i.e. of no “calibration” or lack of (linear) relationship between the transformed ratios, was never significant at 5% level.

3.3. Bronchoprovocation tests in negative tested patients

The mean values of FEV₁, LTB₄, LTC₄, LTD₄, LTE₄, and 8-isoprostane with SD from the five measurements (before the allergen application; after the allergen application = 30 min after baseline measurement; 2, 5, and 24 h after the allergen application) during the negative bronchoprovocation test are given in Table 3.

ANOVA-like analyses were computed for logarithmically transformed measurements with the time factor (five repeated measurements for the markers and FEV₁) in the LME model with random (or individual-specific)

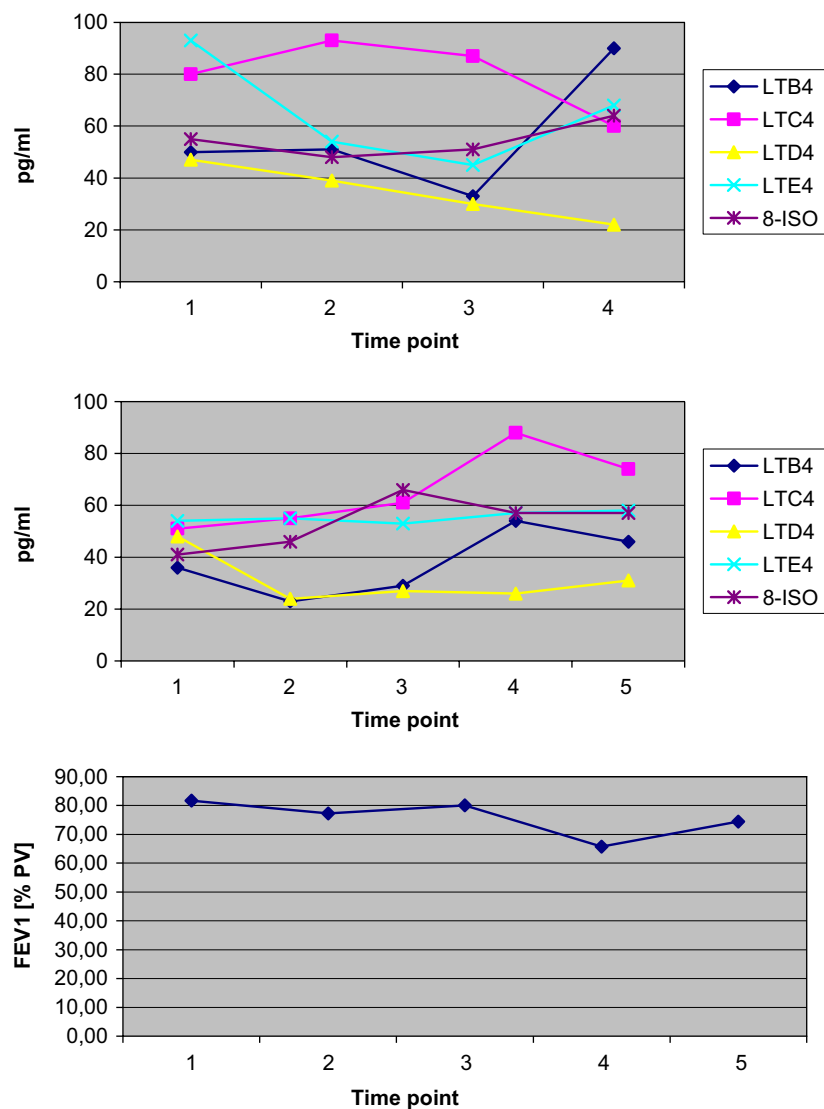


Fig. 5. Patient 3—concentrations of leukotrienes and 8-isoprostane during the monitoring at rest and positive bronchoprovocation test (late asthmatic reaction at time point 4).

intercepts. *P*-values of the tests for null hypothesis that there are no changes over the five measurements were the following: FEV₁ 0.761, LTB₄ 0.4716, LTC₄ 0.0556, LTD₄ 0.903, LTE₄ 0.5365, and 8-isoprostane 0.311. Therefore, no significant changes for FEV₁ or the EBC markers at 5% level (although LTC₄ was very close to the formal borderline) were found.

Fig. 2 compares estimates of the expected values of logs obtained from the LME model for different variables (for each parameter separately).

The relationship between the log transformed ratio of each EBC parameter and FEV₁ was tested too. The *P*-value for the test of null hypothesis about $b = 0$, i.e. of no “calibration” or lack of (linear) relationship between the transformed ratios, was significant only for LTB₄ ($P = 0.0299$) at the times before and 5 h after the test.

3.4. Positive tested patients

Six patients were tested positive. Three persons developed an LAR 5 h after the allergen exposure (tested occupational allergens: flux, 4,4'-diisocyanate + polyol 23, polyurethan + glue) and in three persons an EAR was observed within 0–2 h after the allergen exposure (occupational allergens tested: rye flour, hardener + paste, foam rubber + adhesive).

The number of positive cases is too small for reasonable statistics. That is why the data from the monitoring at rest without provocation and from the positive test are interpreted only visually, as case reports, for each of the six patients (data) and commented on with reference to serious bronchoconstriction.

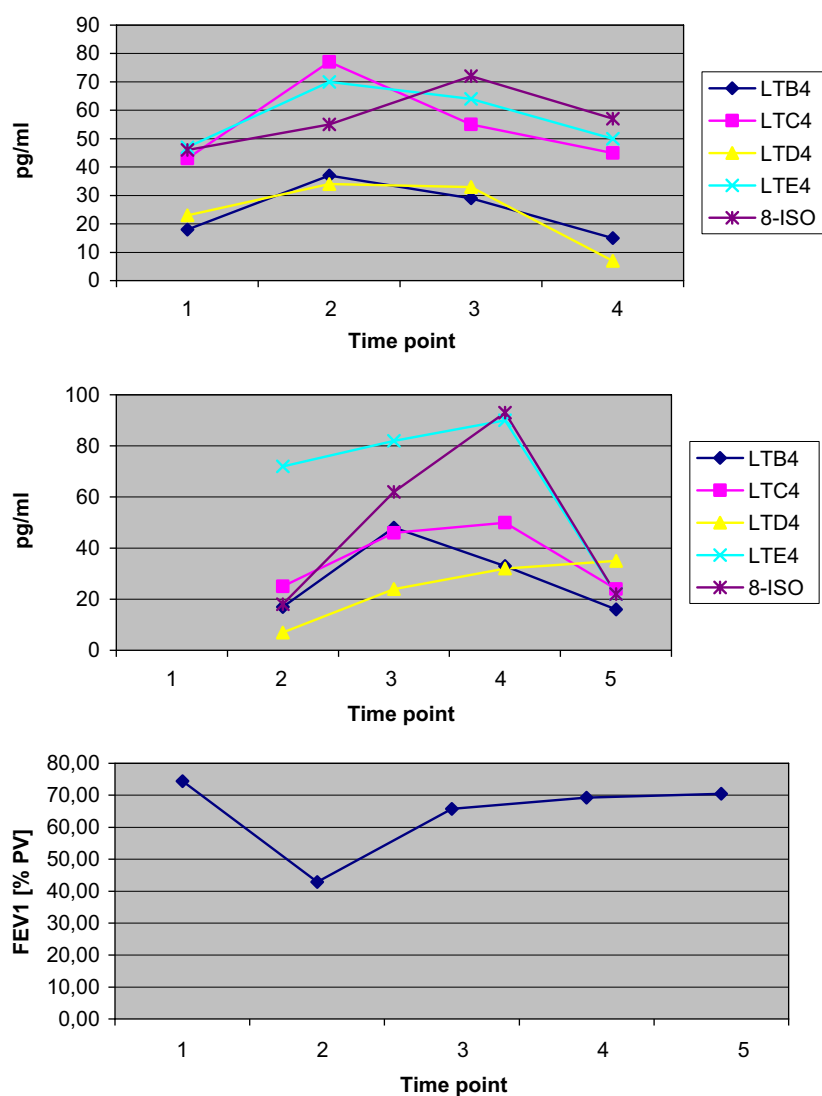


Fig. 6. Patient 4—concentrations of leukotrienes and 8-isoprostane during the monitoring at rest and positive bronchoprovocation test (early asthmatic reaction at time point 2).

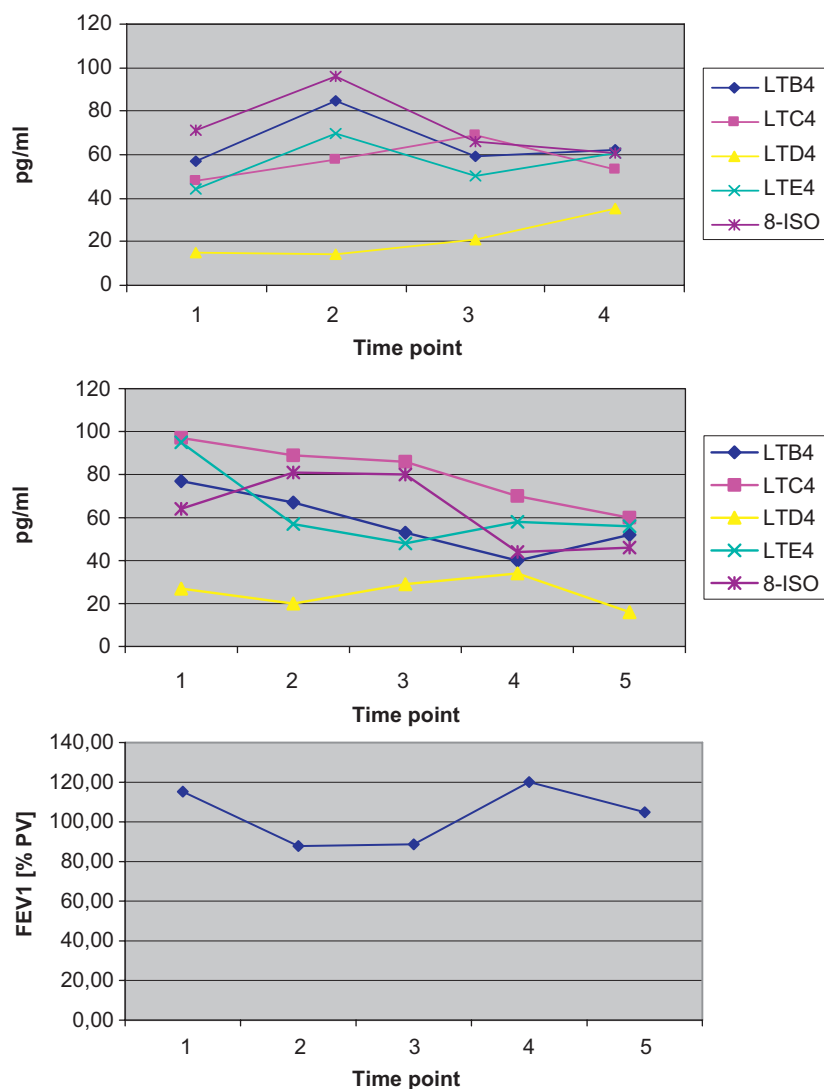


Fig. 7. Patient 5—concentrations of leukotrienes and 8-isoprostane during the monitoring at rest and positive bronchoprovocation test (early asthmatic reaction at time points 2 and 3).

In patient 1, there was no evident (from the figures) fluctuation of the marker concentrations during the monitoring at rest. In the positive test (LAR), some elevation of LTC₄ after the allergen administration was observed, 8-isoprostane was very high in all measurements before and after the test (in contrast to the values obtained at rest) (Fig. 3).

Patient 2 showed no evident fluctuation of the marker concentrations during the monitoring at rest. In the positive test (LAR), some elevation of LTC₄ was detected after the allergen administration and 5 h after the test (two peaks), while LTB₄ was very high in all measurements before and after the test (in contrast to the values obtained at rest) (Fig. 4).

In patient 3, fluctuation in all followed markers was observed at rest. In the positive test (LAR), some elevation of LTC₄ and LTB₄ was recorded at the time of maximum bronchoconstriction (5 h after the test), while

8-isoprostane increased 2 h after the test (before serious bronchoconstriction) (Fig. 5).

In patient 4, there was a tendency toward elevation of the marker concentrations during the midday and afternoon measurements at rest. In the positive test (EAR), elevation of LTC₄, D₄, E₄, and 8-isoprostane was observed 2 h (at the time after serious bronchoconstriction) and 5 h after the test, with LTB₄ elevated only 2 h after the test (Fig. 6).

Patient 5 exhibited a tendency toward elevation of the marker concentrations during the midday (LTB₄, E₄, C₄, 8-isoprostane) and afternoon measurements (LTC₄) at rest. In the positive test (EAR), elevation of 8-isoprostane was observed shortly after and 2 h after the test (at the time of serious bronchoconstriction) (Fig. 7).

In patient 6, there was a tendency toward elevation of LTC₄ and LTE₄ during the midday measurements at

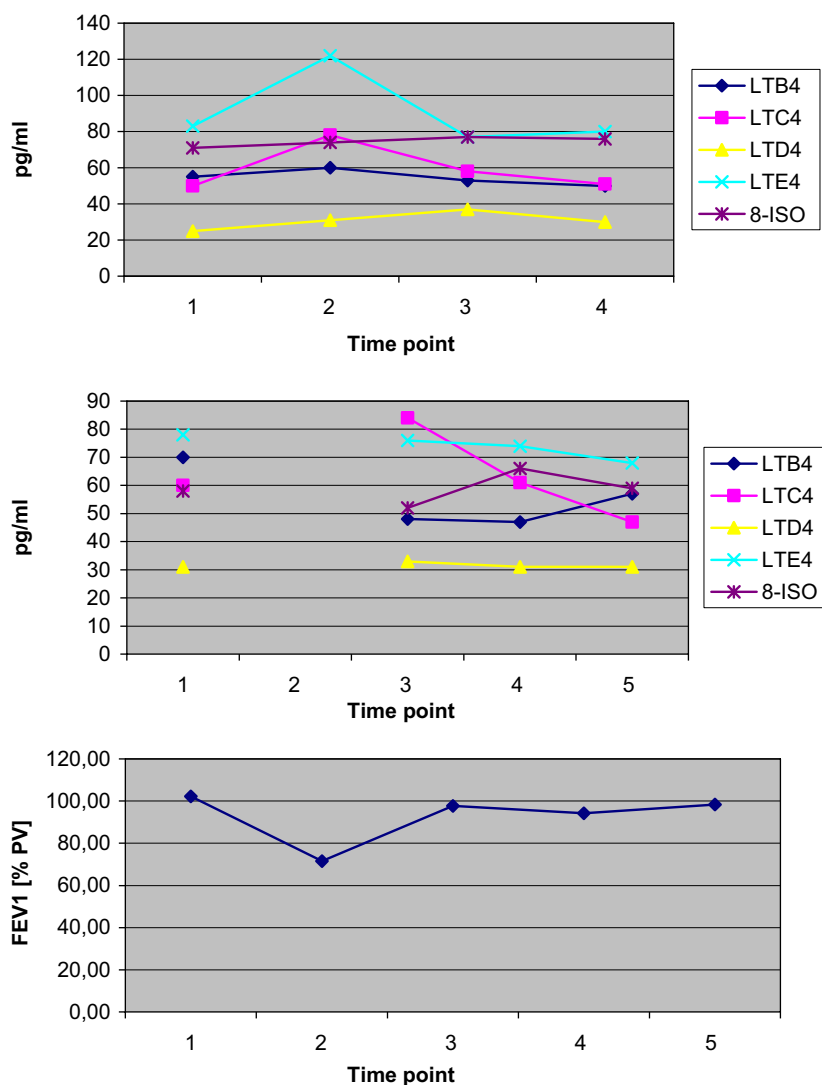


Fig. 8. Patient 6—concentrations of leukotrienes and 8-isoprostane during the monitoring at rest and positive bronchoprovocation test (early asthmatic reaction at time point 2). Notes: LT: leukotriene; 8-ISO: 8-isoprostane; missing values: the analytical device was broken. Intervals of EBC collection at rest (without provocation): time 1 (between 7 a.m. and 10:15 a.m.), time 2 (between 10:30 a.m. and 2 p.m.), time 3 (between 1:30 p.m. and 5:30 p.m.), and time 4 (between 7:00 a.m. and 10:15 a.m. on the next day). Intervals of EBC collection in persons testing positive in the bronchoprovocation test: time 1 (baseline before test), time 2 (after test = 30 min after baseline), time 3 (2 h after test), time 4 (5 h after test), and time 5 (24 h after test).

rest. In the positive test (EAR), elevation of LTC₄ was found 2 h after the test (at the time after bronchoconstriction) (Fig. 8).

4. Discussion and conclusions

Our study was focused on the measurement of EBC leukotrienes and 8-isoprostane concentrations during the bronchoprovocation test. We followed these markers at rest, without provocation, to obtain baseline data. As described in results, we only found significant changes in the 8-isoprostane concentration during the monitoring at rest, with the same persons showing non-significant changes in the EBC markers during the

negative test. The relationship between the transformed ratios of the followed parameters (FEV₁ and EBC parameters) was significant only for LTB₄ before and 5 h after the test. As no other significant relationship was found between any interval and parameter, the importance of the single significant finding is controversial. When the estimates of the log values were presented in the figures, there was a tendency to elevation of the EBC markers shortly after the allergen administration while FEV₁ did not seem to change, but the observed changes were not significant (as it was said previously). As it follows from the results, there was a high interindividual variation in levels of the EBC markers, so it is suggested that they should be used for monitoring of asthma on an individual basis.

Since six persons only were tested positive, no generalization is possible. Moreover, the EAR was observed in three persons and the LAR in the remaining three subjects. The tested allergens were completely different. This problem is generally faced by occupational medicine: there are as many different occupational allergens as there are manufactures in the region. As the marker concentrations varied widely in the positive tests, it was very useful to monitor the remaining study subjects without provocation.

Many studies have detected elevated urinary LTE₄ after allergen application [14–20]. Hanazawa [25] studied changes in the EBC concentrations of leukotrienes in positive bronchoprovocation tests. He has observed increased cysLT in EBC after the allergen challenge test during the late asthmatic response only. Macfarlane [26] has reported significantly elevated cysLT in induced sputum in people with LAR after allergen challenge. In five of six patients with positive provocation tests, there was a tendency toward elevation of LTC₄ in EBC after the test. Elevation of other markers was unique in each person.

Our analytical technique provides a new possibility for the separate detection of cysLT—LTC₄, D₄, and E₄. These parameters are detected together in many studies [27,28]. It posed the problem of low stability of LTC₄ and LTD₄ which was solved by using deuterium-labeled standards. The LC/MS technique is more expensive than the commonly used immunological methods, but has the advantage of being highly specific.

The collection of EBC is a non-invasive technique (in contrast to bronchoalveolar lavage and relatively non-invasive sputum induction). That is why it has a great potential for the future as a diagnostic method. There is a lack of relevant sources for research and diagnosis of occupational asthma. Our study has shown that analysis of leukotrienes in EBC may bring a lot of new information for the evaluation of bronchoprovocation tests with occupational allergens. However, the analysis still needs to be validated before being used in daily routine. Multiple EBC markers can be monitored and different methods are available for EBC analysis (in contrast to NO analysis, which is now used in clinical routine). Studies focused on the events occurring in the airways after the allergen challenge are rather scarce. Therefore, the standardization of the examination and validation of EBC analysis are needed and have been missed for a long time.

This study is preliminary: we did not find any significant changes in EBC leukotrienes either at rest without provocation or in negative bronchoprovocation tests. The data obtained from the positive bronchoprovocation tests should be extended before any generalization can be made. On the basis of our preliminary testing, the focus should be on the monitoring of changes in the EBC parameters after challenge with

one type of occupational allergen because, as it can be seen from our results, no visible similarity between three patients with the same type of asthmatic reaction (EAR or LAR) was observed.

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