Possibilities of Early Diagnosis of Occupational Asthma

Klusáčková P., Pelclová D., Lebedová J.
Charles University in Prague, First Faculty of Medicine,
Department of Occupational Diseases, Prague, Czech Republic

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Mailing Address: Pavlína Klusáčková, MD., Charles University in Prague, First Faculty of Medicine, Department of Occupational Diseases, Na Bojišti 1, 120 00 Prague 2, Czech Republic; Phone: +420 224 964 530; Fax: +420 224 914 570; e-mails: Pavlina.Klusackova@volny.cz, Pavlina.Janu@lf1.cuni.cz

Abstract: Occupational asthma is one of the most frequent occupational diseases of the respiratory tract in developed countries. Moreover, the diagnosis of occupational asthma is difficult because the confirmation of the occupational origin of the disease has an important impact on the career of the employee and many persons must involuntarily leave their work position. To avoid serious consequences, it is necessary to develop new methods which could disclose the incipient occupational asthma earlier than methods available nowadays or support the diagnosis in case of equivocal results (decrease in ventilatory parameters) of the bronchoprovocation tests.

Exhaled breath condensate (EBC) analysis is a new non-invasive method which appears useful in occupational asthma diagnostics. Leukotrienes as obstruction markers and 8-isoprostane as an oxidative stress marker could be analysed from EBC. The concentrations of leukotrienes and 8-isoprostane were described to be elevated in EBC of asthmatic persons. Monitoring of leukotrienes and 8-isoprostane concentration changes in the EBC during the bronchoprovocation tests with allergens could bring new information about the pathophysiological changes in airways during inhalation tests with allergens.

Induced sputum is a relatively non-invasive method which could be used in asthma diagnosis. The monitoring of the sputum cell count (especially changes of eosinophils) has a potential to be used for monitoring of asthma and during allergen challenge tests, too. The elevation of sputum eosinophils was described after allergen tests in several studies.

Introduction

In developed countries, occupational asthma is one of the most frequent occupational diseases of the respiratory tract. Its initiating agent is a substance (allergen) which is present at the workplace. Diagnosis of occupational asthma is difficult because the confirmation of the occupational origin of the disease has a negative impact on the other employee rating and many subjects must leave their present work position. That is why many patients dissimulate and overcome their symptoms. Consequently, they are examined by a physician when the disease is already fully developed.

The long-lasting studies, which followed the patients with occupational asthma after their elimination from the occupational allergen exposure, found out that the disease persists in most patients and the symptoms do not dissolve completely [1]. This could be caused by the late diagnosis of occupational asthma, advanced inflammation in the airways when the mucous membrane became more sensitive towards further allergens exposure. That is why the early diagnosis of occupational asthma is essential. Especially a follow-up of subjects with atopy and of those who are working with offensive allergens is of utmost importance. For this purpose, new methods which could disclose the incipient occupational asthma earlier or support the diagnosis in case of equivocal results of the bronchoprovocation tests, are crucial.

Diagnosis of occupational asthma

Occupational asthma is defined in the Supplement of the Government regulation Nr. 290/1995, i.e. the Czech List of Occupational Diseases, in the chapter III, item 10 as a disease, which develops at the workplace with the exposure to dust or gas particles with allergic or irritative effect. It is supposed that occupational asthma represents about 5–15% of newly diagnosed cases of bronchial asthma [2].

Diagnosis of occupational asthma is based on the verification and the confirmation of bronchial asthma, on the evidence of the relationship between the asthma onset and the work exposure, and on the proof of the sensitization of the patient to the allergen present at the workplace. The verification of occupational asthma can be performed by several ways.

1. The monitoring of the peak expiratory flow parameter (PEF) is the most frequently used way (because of the high safety) in the world. The diurnal variation of PEF higher than 20% during the workplace measurements in comparison with the periods of the elimination from the workplace is considered a positive finding. Another possibility is a monitoring, whether there is a change of the bronchial reactivity during the period of withdrawal from the workplace exposure comparing with the period of exposure at the workplace. In this case at least twofold concentration of inhaled agent to decrease FEV_1 by 20% (PC_{20}) in the period associated with work exposure as regards to the period of withdrawal is needed [3].

2. The specific bronchoprovocation tests with workplace allergens are considered to be the "gold standard" in occupational asthma diagnostics. The occupational allergen exposure can be performed either via direct inhalation of the commercially manufactured standardized allergen (for example flour allergen), or by exposure in the special exposure box with controlled air draw-off mechanism where the patient simulates his/her work at the workplace (for example varnishing). Another possibility is the exposure of the subject at his/her workplace, if it is not possible to find out or to bring the suspected occupational allergen. The positive reaction means the decrease in FEV_1 parameter more than 20% during 24 hours following the inhalation test with regard to the values before test [4].

Sometimes the condition of 20% decrease in FEV_1 parameter after the test is not realized but the value is near 20%. It could be mainly when the testing proceeds without withdrawal of corticosteroid treatment. In these cases some other objective methods could support the final resolution about the positivity of the test. There are two relatively new objective methods which could be helpful in that way- exhaled breath condensate analysis and the analysis of induced sputum.

Exhaled breath condensate

The beginning of exhaled breath condensate (EBC) analysis was in the 18^{th} century, when Lavoisier explored the process of breathing via the exhaled breath analysis. The first works which concerned EBC in relationship to airways disease were

published in the 80^{ies} of the 20th century. They concentrated on the lung surfactant and products of lipid peroxidation. In the 90^{ies} of the 20th century the spectrum of published articles was enlarged by IL-1b, IL-2, IL-6 and TNF. The studies focused on isoprostanes and prostaglandins in asthmatics afterwards appeared, followed by studies of patients with chronic obstructive pulmonary disease (COPD), cystic fibrosis and interstitial lung diseases [5].

The EBC is a rich source of substances (metabolites of arachidonic acid, proteins, hydrogen peroxide, nitro-reactants and many other substances), which reflect the airways environment and offer many possibilities for the diagnostics and monitoring of various respiratory diseases [5]. Mediators from the airways are released into the exhaled breath, which then condensates in the cooled sampling tube. The collection of the EBC is a simple, non-invasive method which can be performed in children and seriously ill patients and can be repeated several times over [6]. It is supposed that aerosol particles of the EBC reflect the composition of the bronchoalveolar fluid [5].

Collection of EBC samples

EBC samples could be collected using various devices. Generally, they are composed from the mouthpiece with a one-way valve connected to the sampling device, which is cooled either by ice or by liquid nitrogen. The commercially manufactured Eco-Screen (Jaeger, Hoechberg, Germany) is an electricity cooled system. The examined person breathes into the system through the mouthpiece, connected to a valve block, in which the inspiratory and expiratory air is separated. The air is cooled by the countercurrent system in a collecting part of the system, which is composed of the lamellar condenser and the sample collection vial from the inert material. The liquid condenses on the inner wall of the condenser and drops into the vial.

Contamination of the EBC samples

During the sampling, the EBC could be contaminated by substances from the environment or by endogenous substances from the mouth and upper airways, but the risk of endogenous contamination is small because the laminar circulation is not sufficient enough for the aerosol formation. The surface of the upper airways is smaller comparing to the lower airways, therefore the incidental contamination could be considered as not significant. On the contrary, some substances are present in high concentrations in saliva. Consequently, it is recommended to control the saliva contamination of the EBC by the measurement of α-amylase [5].

EBC analysis

Many analytic methods are used nowadays for the assessment of the substances in the EBC. It is possible to use the immunologic methods (EIA, ELISA, RIA) preferred for the financial reasons, the photometric methods (fluorescence,

chemiluminescence), the high-pressure liquid chromatography (HPLC), or the combination of mass spectrometry with liquid or gas chromatography (LC/MS, GC/MS). LC/MS or GC/MS appeared the most convenient for the leukotrienes and 8-isoprostane analysis because of their high specificity and ability to detect very low concentration of these markers (pg/ml). The disadvantage of these methods is a relatively high cost. Very sensitive ionization techniques are used too (negative chemical ionization), eventually monitoring of some specific ions in the spectrum of the measured substances (SIM – Selected Ion Monitoring). The decrease of the detection limit is possible because of the samples pre-treatment by lyophilisation or Solid Phase Extraction (SPE) [6].

Leukotrienes in EBC

Leukotrienes are metabolites of arachidonic acid. Arachidonic acid is 20-carbonic polyunsaturated fat acid that can be biotrasformed both in enzymatic and non-enzymatic ways. The two main metabolic ways are dependent of the enzymes cyclooxygenase (COX) and 5-lipoxygenase (5-LO). Leukotrienes originate from the 5-lipoxygenase pathway. The initiation of the leukotrienes biosynthesis is dependent on the agonist stimulation (allergen associated IgE binding to the mast cell membrane), hydrolysis of arachidonic acid from the phospholipide membrane by phospholipase A_2 and the interaction of 5-LO with the 5-LO activating protein (FLAP). 5-LO catalyses the conversion of arachidonic acid to 5-hydroperoxyeicosatetraenoic acid (HPETE), which is converted to hydroxyeicosatetraenoic acid (HETE) or to the unstable epoxide – leukotriene A_4 (LTA_4). In alveolar macrophages, monocytes and neutrophils, LTA_4 is metabolized by hydrolysis to LTB_4. In mast cells, eosinophils and basophils (the main inflammatory allergic cells) the conversion of LTA_4 to leukotriene C_4 (LTC_4) takes place via LTC_4 synthase. LTC_4 is extracellularly metabolized by γ-glutamyltranspeptidase to leukotriene D_4 (LTD_4) which is biotransformed by dipeptidase to leukotriene E_4 (LTE_4). LTC_4, LTD_4 and LTE_4 are known as cysteinyl leukotrienes (cys-LT) or formerly slow reacting substance of anaphylaxis (SRSA). LTE_4 is the final metabolite of this leukotrienes line and is excreted in the urine. Its concentration in urine could be detected as the whole amount of the total body cys-LT production. The leukotrienes effect is connected with the G protein receptors BLT (for LTB_4) and receptors $cysLT_1$ and $cysLT_2$ (for cys-LT) [7]. $CysLT_1$ receptor is present on the pulmonary macrophages, smooth muscle cells, eosinophils, basophils, monocytes and mast cells, B lymphocytes and CD34 cell precursors; it was detected in the spleen, colon, placenta, pancreas, prostate, heart, brain, kidneys, liver and fat tissue. $CysLT_2$ receptor is present on the Purkyně heart fibres, adrenal gland cells and brain cells, eosinophils and macrophages [8]. During in-vitro monitoring of the contractile capacity, approximately the same potential of LTC_4 and LTD_4 was described to initiate the smooth muscle airways constriction. The potential of LTE_4 was lower [9]. Cys-LT

could be considered as airways obstruction mediators [10]. $CysLT_2$ receptor is associated with the pulmonary vasoconstriction in the lungs [7].

Cys-LT mediate bronchoconstriction, increases bronchial hyperreactivity, plasma exsudation, they support Th2 cell response, suppress Th1 response, evoke eosinophils' infiltration and degranulation, hyperplasia of mucous cells, mucus hypersecretion, pulmonary basal corpuscles inhibition, smooth muscles hyperplasia, epithelial cells proliferation, they increase collagen deposition, and they exert vasodilatation or vasoconstriction.

The main activities of LTB_4 are chemotaxis of neutrophils, support of IgE synthesis and nuclear transcription.

Cys-LT production was in the past monitored only by the urine LTE_4 concentration. This concentration reflects the total body leukotrienes production, from which the airways production represents only a part. The EBC collection enables monitoring of the leukotrienes concentration changes namely from the airways and involves not only LTE_4 but also the main contractile leukotrienes LTC_4 and LTD_4 [9]. Based on the fact that cys-LT participate on the smooth muscle contraction, the concentration of LTE_4 as a main metabolite of cys-LT in urine was measured at rest and during non-specific and specific bronchoprovocation tests with occupational allergens. The concentration of LTE_4 in urine was monitored before and during bronchoprovocation tests with allergens and after aspirin administration in aspirin-sensitive asthmatics. After allergen exposure the elevation of LTE_4 in urine was found in atopic asthmatics [11]. Taylor [12] and Smith [13] described elevation of LTE_4 in urine during three hours after allergen inhalation. Manning [14] studied concentrations of LTE_4 during the early and late allergic reaction. During the early response the level of bronchoconstriction correlated with the elevation of LTE_4 concentration in urine; however during the late response the elevation of LTE_4 concentration was not significant. In contrast, O'Sullivan [15] described the elevation of LTE_4 concentration in urine both during the early and the late allergic reaction after the allergen inhalation. A significant elevation of LTE_4 concentration was described in aspirin sensitive asthmatics after aspirin administration in comparison to the level before the application [11, 16, 17].

Ono [18] described the elevation of cys-LT concentrations in the EBC after specific bronchoprovocation tests with allergen during the early allergic response together with increased concentration of LTE_4 in urine.

LTB_4 and cys-LT were detected in the EBC of healthy subjects, and comparing with them they were elevated in adult asthmatics with stable moderate to severe asthma [19]. In persons with aspirin sensitive asthma not treated by corticosteroids, the concentrations of cys-LT in the EBC were significantly higher than in aspirin-tolerating asthmatics not treated with corticosteroids. On the contrary, the cys-LT concentrations did not significantly differ between the groups of aspirin-tolerating asthmatics treated and not-treated by corticosteroids. Cys-LT

concentrations were higher in asthmatics than in healthy persons [20]. Mondini [21] found significantly higher LTE_4, LTB_4 and 8-isoprostane concentrations in asthmatic children with atopy (both treated and not-treated by corticosteroids) in contrast to the healthy persons. On the other hand, LTB_4 was found elevated in persons with COPD. Montuschi [22] described increased LTB_4 in the EBC in smoking persons with COPD both treated and not-treated by corticosteroids in comparison to healthy persons.

8-isoprostane in EBC

Isoprostanes originate from the non-enzymatic peroxidation of arachidonic acid [23]. F_2-isoprostanes are considered the best biomarkers of the oxidative stress and lipids' peroxidation in vivo as regards to their stability, specificity in endogenous peroxidation and relatively easy detection in biological samples [24]. Many their effects on the respiratory system are known, i.e. contraction of bronchial smooth muscles (in vitro) [25], obstruction of the airways with the plasma exsudation in guinea-pigs in vivo [26]. In addition, isoprostanes as markers of the oxidative stress increase in subjects with interstitial lung diseases, what points to their possible utilization as potential mediators of the activity of certain pulmonary diseases caused by oxidative stress, such as pneumoconioses [27].

Increased 8-isoprostane concentration in the EBC was found in persons with stable asthma (in contrast to healthy persons). Moreover, its elevation correlated with the asthma severity and the degree of inflammation [28] in asthmatics with both aspirin-sensitive and aspirin-tolerating asthma, and also in asthmatic children both treated and not-treated by corticosteroids [29, 30]. There results support the fact that 8-isoprostane as a marker of oxidative stress is elevated in adults and children with asthma and this increase is relatively independent on the corticosteroid treatment [28].

8-isoprostane was increased in smokers and ex-smokers with stable COPD in comparison with healthy smokers and non-smokers [30]. It was described that during exacerbations of COPD 8-isoprostane concentration increased and subsequently decreased after the antibiotic treatment [31]. No correlation was found between ventilatory parameters and 8-isoprostane in the EBC either in asthmatics or COPD patients [30], but negative correlation was found for FEV_1 in patients with cystic fibrosis [32].

Utilization of EBC analysis in occupational asthma diagnostics

In several above mentioned studies, LTE_4 in urine and cys-LT in EBC were described elevated after bronchoprovocation tests with allergens. Cys-LT are considered as markers of airways obstruction so their elevation could support the positive result of the bronchoprovocation test in case of borderline decrease of ventilatory parameters (not more than 20% decrease of FEV_1 after the test). This could be crucial in the occupational asthma assessment.

In our department a pilot study with 47 persons who underwent bronchoprovocation tests with occupational allergens was performed. In 3 patients with the late asthmatic reaction LTC_4 was elevated before and/or at the time of the maximum decrease in FEV_1. In 3 patients with an early asthmatic reaction no important changes were observed [33]. LTC_4 could be a helpful marker in occupational asthma diagnostics in our conditions but there is a need of more trials before any conclusion is done because the tested group was very small.

Induced sputum

Sputum induction (sputum obtained using inhalation of saline solutions) is not a completely new method in medicine. This method was used in the 50^{ies} of the 20th century by Bickerman for obtaining sputum which was used in the lung cancer diagnostics. In the 90^{ies} of the 20th century the method of the induced sputum was modified by Pin and Fahy and the epoch of using of the induced sputum in diagnostics and treatment of bronchial asthma started [34]. The induced sputum analysis can bring the data both about cells and inflammatory markers (analysis of sputum supernatant). The information about the inflammation in the lower respiratory tract can be obtained also from the bronchoalveolar lavage fluid, bronchial wash or bronchial biopsy. However, these examinations are invasive and less tolerated by the patients. In the comparison of these methods a good correlation was found between eosinophils in induced sputum and in bronchoalveolar lavage fluid, unlike with eosinophils from the bronchial biopsy [35].

The idea of sputum induction results from the fact that not all persons are able to produce sputum spontaneously. That is why sputum was induced using the inhalation of the hypertonic saline solutions (sodium chloride, NaCl). The most frequently recommended concentration is 4.5%, because it is produced commercially [36]. The inhaled saline concentration can be subsequently increased, too, if it is well tolerated by the patient (the most frequent combination is 3%, 4%, and 5% NaCl). This schema is feasible for patients with increased bronchial hyperresponsiveness where increased caution is required. In patients with higher risk of bronchospasm it is possible to start the inhalation with the isotonic physiological saline solution (0.9% NaCl). The highest described NaCl concentration used for sputum induction was 7% [37]. The hypertonic saline is more effective in sputum induction than the isotonic saline; however the cell distribution in sputum is not significantly different [38]. Another substance which was used for sputum induction was uridine-5'-phosphate. A lower decrease of ventilatory parameters and a greater amount of sputum were described after using this substance in comparison with NaCl [39].

Ultrasound nebulisers are mainly recommended for sputum induction because most of other nebulisers do not have an adequate output. The required output of the nebuliser is approximately 1 ml/min [36].

During sputum induction it is necessary to monitor ventilatory parameters (the parameter FEV_1 is usually monitored during sputum induction.). In persons with the increased bronchial reactivity the hypertonic saline solution could cause a decrease in ventilatory parameters and bronchoconstriction. These undesirable effects could be prevented by the application of salbutamol (200–400 μg) or other β_2-agonist before saline inhalation. Induced sputum has already been analysed in many trials and the effect of pre-treatment by β_2-agonists on the sputum cell count has been studied. It was not confirmed that the pre-treatment by salbutamol significantly influenced the sputum cell count [38, 40]. The effect of salbutamol pre-treatment on sputum ECP concentration was not found either, contrary to histamine concentrations which decreased after this pre-treatment [40].

The induced sputum could be processed as a whole sample (with saliva) or as selected sputum (selected plaques of sputum). Both methods have their advantages and disadvantages. The non-selected sputum method is relatively faster but the sample could be more contaminated by the epithelium cells from mouth. The selected sputum method is more difficult for the laboratory processing but the samples are better for the visual microscope cell readout.

The induced sputum cell count contributes to the diagnosis of respiratory diseases. The total sputum cell count increases in the uncontrolled asthma, in smokers and due to bacterial infections [41, 42]. Bronchial asthma or eosinophilic bronchitis and allergen exposure in allergen sensitive persons increase the number of eosinophils in sputum. Up to 80% of asthmatics not-treated by corticosteroids and up to 50% of steroids treated asthmatics with asthmatic symptoms have increased eosinophils in sputum [43, 44, 45]. On the other hand, in chronic bronchitis, COPD, in smokers, in respiratory infections, in ozone and other air pollutants exposure, in endotoxins exposure and in non-eosinophilic steroid-resistant bronchial asthma, the neutrophils become elevated in sputum. Increased sputum lymphocytes were described in sarcoidosis and pneumonia caused by *Chlamydia* [46].

Although induced sputum method was originally developed for the detection of the infectious agents, in last years this method appeared to be very useful for the study of asthma and COPD. The study of Pin demonstrated increased number of eosinophils and metachromatic cells in sputum count of asthmatics contrary to the healthy subjects [47]. Fahy found that the acellular part of sputum from asthmatics contained an increased concentration of ECP and albumin in comparison to healthy subjects [48, 49]. The increased number of eosinophils in sputum before the start of treatment is also a good predictor of fruitfulness of corticosteroids treatment [50]. Chlumský [51] described a correlation between the level of the bronchial obstruction and percentage of eosinophils in sputum. The presence of eosinophils in sputum in patients treated with inhaled corticosteroids pointed on the chronic persisting inflammation which deficiently answered to the inhaled corticosteroids treatment and frequently required the treatment with systemic corticosteroids [51].

Utilization in occupational asthma diagnosis

Several studies describing utilization of induced sputum in occupational asthma diagnostics were published. Lemiere [52] studied changes of cell parameters in induced sputum during specific bronchoprovocation tests and described a significant increase of the eosinophils and neutrophils, and a decrease of macrophages percentage in sputum 7 hours after the exposure to occupational allergens (high molecular weight (HMW) and low molecular weight (LMW) allergens) in patients who positively answered to the specific bronchoprovocation test. In subjects with a negative bronchoprovocation test a significant increase of eosinophils in 2 from 14 persons was also found, but this increase was not as high as after a positive test. Lemiere [53] examined also the changes in sputum after gradually increasing allergen concentrations in patients with previously diagnosed occupational asthma concurrently with the ventilatory parameters monitoring. The increase of sputum eosinophils preceded a significant decrease of ventilatory parameters (bronchoconstriction) and was more pronounced in patients exposed to LMW allergens. Obata [54] described a significant increase of the sputum eosinophil percentage 6 and 24 hours after the positive bronchoprovocation test in 9 persons exposed to the red cedar (plicatic acid as LMW allergen). After a negative challenge test a significant increase of sputum eosinophils was found in 3 from 8 persons. Changes in induced sputum were also monitored before and during the exposure to suspected allergens at the workplace (4 weeks of exposure) and after elimination from the workplace allergens exposure (4 weeks outside the workplace). Sputum induction was performed up to 48 hours after the last workplace exposure and after 4 weeks outside the workplace. The patients with occupational asthma had significantly increased sputum eosinophils and sputum ECP during the workplace exposure contrary to the period of their elimination from the workplace [55].

In our department the non-selected and selected method of sputum processing was tested. If the method of non-selected sputum was used, we did not found the elevation of sputum eosinophils neither after the positive specific test with the occupational allergen or after negative tests [56]. Moreover many samples of sputum were highly contaminated by the epithelium cells from mouth. In comparison the selected sputum method was more effective. In the pilot study with 23 persons we found the significant increase of sputum eosinophils in the group of 7 persons with positive result of the bronchoprovocation test with the occupational allergen. Moreover in two persons with the negative result of the test (the decrease in FEV_1 did not reach 20%) the significant elevation of eosinophils was found too [57].

The monitoring of induced sputum shows that in most of the sensitised people the sputum eosinophils increase due to the exposure to the occupational allergen. In the literature this elevation is mainly described for LMW allergens. It is not

evident why some allergens cause sputum eosinophils elevation and some not despite the evident bronchoconstriction after the allergen application.

The elevation of sputum eosinophils supports the positive results of the allergen bronchoprovocation test. In persons with the negative bronchoprovocation test (not significant decrease of FEV_1), the elevation of sputum eosinophils after the test supports the idea of the continuing in the allergen exposure – the positive result of the test (significant decrease of FEV_1) could be reached after the repeated exposure to the occupational allergen.

Conclusion

Two promising methods which could bring new information to asthma (not only occupational) diagnostics were described in this review article. The contribution of these methods to the investigation of asthma pathophysiology was confirmed by many studies. Obviously, these methods could also be used in occupational asthma diagnostics which is particularly difficult. The effect of exposure to specific allergens both on the sputum cell count and on the concentration of leukotrienes and 8-isoprostane in the EBC could contribute to a better knowledge of the pathophysiology of occupational asthma.

References

1. KLUSACKOVA P., PELCLOVA D., LEVEDOVA D. J., MARECKOVA H., BRABEC M.: Occupational asthma after withdrawal from the occupational allergen exposure. *Industrial Health* 44: 629–638, 2006.
2. BERNSTEIN J. A., BERNSTEIN D. I.: Occupational asthma. Diagnostic approaches and treatment. In: Environmental asthma. Bush R. K., Marcel Dekker, Inc., New York, Basel, 2001, 265–284.
3. NOVOTNÁ B., BRHEL P.: Profesionální bronchiální astma (in Czech). *Alergie* 2: 120–126, 2000.
4. SATINSKÁ J.: Testy bronchiální reaktivity. In: Funkční vyšetření plic (in Czech). Fišerová J., Chlumský J., Satinská J. (eds), Geum, Praha, 2003, 37–55.
5. ČÁP P., PEHAL F., PETRŮ V., MUSIL J.: Kondenzát vydechovaného vzduchu a možnosti jeho vyšetření (in Czech). *Alergie* 4: 275–280, 2001.
6. BARNES P. J.: Exhaled breath condensate: A new approach to monitoring lung inflammation. In: New perspectives in monitoring lung inflammation: Analysis of exhaled breath condensate. Montuschi P., CRC Press, Boca Raton, 2005, 1–10.
7. DWORSKI R., PEEBLES R. S. JR., SHELLER J. R.: The role of leukotrienes and prostanoids in airway inflammation. In: New perspectives in monitoring lung inflammation: Analysis of exhaled breath condensate. Montuschi P., CRC Press, Boca Raton, 2005, 149–166.
8. KOPŘIVA F.: Eikosanoidy. In: Leukotrieny (in Czech). Kopřiva F., Jessenius Maxdorf, Praha, 2005, 25–43.
9. DRAZEN J.: Leukotrienes as mediators of airway obstruction. *Am. J. Respir. Crit. Care Med.* 158: 193–200, 1998.
10. DAHLEN S. E., KUMLIN M., GRANSTROM E., HEDQVIST P.: Leukotrienes and other eicosanoids as mediators of airway obstruction. *Respiration* 50(Suppl. 2): 22–29, 1986.
11. KUMLIN M., DAHLEN B., BJORCK T., ZETTERSTROM O., GRANSTROM E., DAHLEN S. E.: Urinary excretion of leukotriene E4 and 11-dehydro-thromboxane B2 in response to bronchial provocations with allergen, aspirin, leukotriene D4, and histamin in asthmatics. *Am. Rev. Respir. Dis.* 146: 96–103, 1992.

12. TAYLOR G. W., TAYLOR I., BLACK P., MALTBY N. H., TURNER N., FULLER R. W., DOLLERY C. T.: Urinary leukotriene E4 after antigen challenge and in acute asthma and allergic rhinitis. *Lancet* 1(8638): 584–588, 1989.
13. SMITH C. M., CHRISTIE P. E., HAWKSWORTH R. J., THIEN F., LEE T. H.: Urinary leukotriene E4 levels after allergen and exercise challenge in bronchial asthma. *Am. Rev. Respir. Dis.* 144: 1411–1413, 1991.
14. MANNING P. J., ROKACH J., MALO J. L., ETHIER D., CARTIER A., GIRARD Y., CHARLESON S., O'BYRNE P. M.: Urinary leukotriene E4 levels during early and late asthmatic responses. *J. Allergy Clin. Immunol.* 86: 211–220, 1990.
15. O'SULLIVAN S., ROQUET A., DAHLEN B., DAHLEN S., KUMLIN M.: Urinary excretion of inflammatory mediators during allergen-induced early and late phase asthmatic reactions. *Clin. Exp. Allergy* 28: 1332–1339, 1998.
16. KNAPP H. R., SLADEK K., FITZGERALD G. A.: Increased excretion of leukotriene E4 during aspirin-induced asthma. *J. Lab. Clin. Med.* 119: 48–51, 1992.
17. CHRISTIE P. E., TAGARI P., FORD-HUTCHINSON A. W., CHARLESSON S., CHEE P., ARM J. P., LEE T. H.: Urinary leukotriene E4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am. Rev. Respir. Dis.* 143: 1025–1029, 1991.
18. ONO E., MITA H., TANIGUTCHI M., HIGASHI N., TSUBURAI T., HASEGAWA M., MIYAZAKI E., KUMAMOTO T., AKIYAMA K.: Increase in inflammatory mediator concentrations in exhaled breath condensate after allergen inhalation. *J. Allergy Clin. Immunol*. published online 14 July 2008: http://www.jacionline.org/article/S0091-6749(08)01130-5/fulltext
19. HANAZAWA T., KHARITONOV S. A., BARNES P. J.: Increased nitrotyrosine in exhaled breath condensate in patients with asthma. *Am. J. Respir. Crit. Care Med.* 162: 1273, 2000.
20. ANCZAK A., MONTUSCHI P., KHARITONOV S., GORSKI P., BARNES P. J.: Increased exhaled cysteinyl-leukotrienes and 8-isoprostane in aspirin-induced asthma. *Am. J. Respir. Crit. Care Med.* 166: 301–306, 2002.
21. MONDINI C., CIABATTONI G., KOCH P., PISTELLI R., TROVÉ A., BARNES P. J., MONTUSCHI P.: Effects of inhaled corticosteroids on exhaled leukotrienes and prostanoids in asthmatic children. *J. Allergy Clin. Immunol.* 114: 761–767, 2004.
22. MONTUSCHI P., KHARITONOV S. A., CIABATTONI G., BARNES P. J.: Exhaled leukotrienes and prostaglandins in COPD. *Thorax* 58: 585–588, 2003.
23. MORROW J. D., HILL K. E., BURK R. F., NAMMOUR T. M., BADR K. F., ROBERTS L. J.: A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical catalyzed mechanism. *Proc. Natl. Acad. Sci. USA* 87: 9383–9387, 1990.
24. ROBERTS L. J., MORROW J. D.: Measurement of F2-isoprostanes as an index of oxidative stress in vivo. *Free Radic. Biol. Med.* 28: 505–513, 2000.
25. KAWIKOVA I., BARNES P. J., TAKAHASHI T., TADJKARIMI S., YACOUB M. H., ELVISI M. G.: 8-epi-PGF2α, a novel noncyclooxygenase-derived prostaglandin, constricts airways in vitro. *Am. J. Respir. Crit. Care Med.* 153: 590–596, 1996.
26. OKAZAWA A., KAWIKOVA I., CUI Z. H., SKOOGH B. E., LOTVALL J.: 8-Epi-PGF2alpha induces airflow obstruction and airway plasma exsudation in vivo. *Am. J. Respir. Crit. Care Med.* 155: 436–441, 1997.
27. PELCLOVÁ D., FENCLOVÁ Z., KACER P., NAVRÁTIL T., KUZMA M., LEBEDOVÁ J., KLUSÁČKOVÁ P.: 8-isoprostane and leukotrienes in exhaled breath condensate in Czech subjects with silicosis. *Ind. Health* 45: 766–74, 2007.
28. MONTUSCHI P., CORRADI M., CIABATTONI G., NIGHTINGALE J., KHARITONOV S. A.,

BARNES P. J.: Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am. J. Respir. Crit. Care Med.* 160: 216–20, 1999.
29. BARALDI E., GHIRO L., PIOVAN V., CARRARO S., CIABATTONI G., BARNES P. J., MONTUSCHI P.: Increased exhaled 8-isoprostane in childhood asthma. *Chest* 124: 25–31, 2003.
30. MONTUSCHI P., COLLINS J. V., CIABATTONI G., LAZZERI N., CORRADI M., KHARITONOV S. A., BARNES P. J.: Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. *Am. J. Respir. Crit. Care Med.* 162: 1175–1177, 2000.
31. BIERNACKI W. A., KHARITONOV S. A., BARNES P. J.: Increased leukotriene B4 and 8-isoprostane in exhaled breath condensate of patients with exacerbations of COPD. *Thorax* 58: 294–298, 2003.
32. MONTUSCHI P., KHARITONOV S. A., CIABATTONI G., CORRADI M., RENSEN L., GEDDES D. M., HODSON M. F., BARNES P. J.: Exhaled 8-isoprostane as a new non-invasive biomarker of oxidative stress in cystic fibrosis. *Thorax* 55: 205–209, 2000.
33. KLUSACKOVA P., LEBEDOVA J., KACER P., KUZMA M., BRABEC M., PELCLOVA D., FENCLOVA Z., NAVRATIL T.: Leukotrienes and 8-isoprostane in exhaled breath condensate in bronchoprovocation tests with occupational allergens. *Prostaglandins Leukot. Essent. Fatty Acids* 78: 281–292, 2008.
34. HARGREAVE F. E., LEIGH R.: Induced sputum, eosinophilic bronchitis, and chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 160: 53–57, 1999.
35. GROOTENDORST D. C., SONT J. K., WILLEMS L. N., KLEIN-NELEMANS J. C., VAN KRIEKEN J. H., STERK P. J.: Comparison of inflammatory cell count in asthma: induced sputum versus bronchoalveolar lavage and bronchial biopsies. *Clin. Exp. Allergy* 27: 769–779, 1997.
36. PAGGIARO P. L., CHANEZ P., HOLZ O., IND P. W., DJUKANOVIC R., MAESTRELLI P., STERK P. J.: Sputum induction. *Eur. Respir. J.* 20(Suppl. 37): 3s–8s, 2002.
37. PAGGIARO P., SPANEVELLO A., BACCI A.: Sputum induction: methods and safety. In: An atlas of induced sputum. DJUKANOVIC R., STERK P. J., The Panthenon Publishing Group, London, 2004, 11–19.
38. POPOV T. A., PIZZICHINI M. M., PIZZICHINI E., KOLENDOWICZ R., PUNTHAKEE Z., DOLOVICH J., HARGREAVE F. E.: Some technical factors influencing he induction of sputum for cell analysis. *Eur. Respir. J.* 8: 559–565, 1995.
39. TAMAOKI J., KONDO M., KURODA H., TOSHIBA K., TAKEYAMA K., NAKATA J., NAGAI A.: Validity and safety of sputum induction by inhaled uridin-5´-triphosphate. *Am. J. Respir. Crit. Care Med.* 164: 378–381, 2001.
40. CIANCHETTI S., BACCI E., RUOCCO L., BARTOLI M. L., CARNEVALI S., DENTE F. L., DI FRANCO A., GIANNINI D., SCUOTRI L., VAGAGGINI B., PAGGIARO P. L.: Salbutamol pretreatment does not change eosinophil percentage and eosinophilic cationic protein concentration in hypertonic saline-induced sputum in asthmatic subjects. *Clin. Exp. Allergy* 29: 712–718, 1999.
41. PIZZICHINI E., PIZZICHINI M. M. M., EFTHIMIADIS A., EVANS S., MORFIA M. M., SQUILLACE D., GLEICH G., DOLOVICH J., HARGREAVE F. E.: Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid phase measurement. *Am. J. Respir. Crit. Care Med.* 154: 308–317, 1996.
42. EFTHIMIADIS A., PIZZICHINI E., PIZZICHINI M. M. M., HARGREAVE F. E.: Sputum examination for indices of airway inflammation: Laboratory procedures, Astra Draco, Lund, Sweeden, 1997, 1–29.
43. PAVORD I. D., PIZZICHINI M. M., PIZZICHINI E., HARGREAVE F. E.: The use of induced sputum to investigate airway inflammation. *Thorax* 52: 498–501, 1997.
44. LOUIS R., LAU L. C., BRON A. O., ROLDAAN A. C., RADERMECKER M., DJUKANOVIC R.: The relationship between airways inflammation and asthma Severiny. *Am. J. Respir. Crit. Care Med.* 161: 9–16, 2000.

45. PAVORD I. D., STERK P. J., HARGREAVE F. E., KIPS J. C., INMAN M. D., LOUIS R., PIZZICHINI M. M. M., BEL E. H., PIN I., GROOTENDORST D. C., PAREMESWARAN K., DJUKANOVIC R.: Clinical applications of assessment of airway inflammation using induced sputum. *Eur. Respir. J.* 20 (Suppl. 20): 40s–43s, 2002.
46. JARAYJAM L., PARAMESWARAN K., SEARS M. R., HARGREAVE F. E.: Induced sputum cell counts: their usefulness in clinical practise. *Eur. Respir. J.* 16: 150–158, 2000.
47. PIN I., GIBBON P. G., DOLOVICH J., KOLENDOWICZ R., GIRGIS-GABARDO A., DENBURG J. A., HARGREAVE F. E., DOLOVICH J.: Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 47: 25–29, 1992.
48. FAHY J. V., LIU J., WONG H., BOUSHEY H. A.: Cellular and biochemical analysis of induced sputum from asthmatics and from healthy subjects. *Am. Rev. Respir. Dis.* 147: 1126–1131, 1993.
49. LOUIS R. A., CHANEZ P.: Induced sputum studies in asthma. In: An atlas of induced sputum. DJUKANOVIC R., STERK P. J., The Panthenon Publishing Group, London, 2004, 53–70.
50. PIZZICHINI E., PIZZICHINI M. M. M., GIBSON P., PARAMESWARAN K., GLEICH G. J., BERMAN L., DOLOVICH J., HARGREAVE F. E.: Sputum eosinophilia predicts benefit from prednisone in smokers with chronic obstructive bronchitis. *Am. J. Respir. Crit. Care Med.* 158: 1511–1517, 1998.
51. CHLUMSKÝ J., POKORNÁ H.: Buněčné parametry indukovaného sputa jako neinvazivní znak zánětu dýchacích cest u pacientů s astmatem: absence korelace mezi maximálním poklesem FEV1 a PEF po inhalaci hypertonického roztoku NaCl a zastoupením eosinofilních leukocytů v indukovaném sputu (in Czech). *Alergie* 2: 108–111, 2001.
52. LEMIERE C., CHABOILLEZ S., MALO J. L., CARTIER A.: Changes in sputum cell counts after exposure to occupational agents: What do they mean? *J. Allergy Clin. Immunol.* 107: 1063–1068, 2001.
53. LEMIERE C., CHABOILLEZ S., TRUDEAU C., TAHA R., MAGHNI K., MARTIN J. G., HAMID Q.: Characterization of airway inflammation after repeated exposures to occupational agents. *J. Allergy Clin. Immunol.* 106: 1163–1170, 2000.
54. OBATA H., DITTRICK M., CHAN H., CHAN-YEUNG M.: Sputum eosinophils and exhaled nitric oxide during late asthmatic reaction in patients with western red cedar asthma. *Eur. Respir. J.* 13: 489–495, 1999.
55. LEMIERE C., PIZZICHINI M. M. M., BALKISSOON R., CLELLAND L., EFTIMIADIS A., O'SHAGHNESSY D., DOLOVICH J., HARGREAVE F. E.: Diagnosing of occupational asthma: use of induced sputum. *Eur. Respir. J.* 13: 482–488, 1999.
56. KLUSÁČKOVÁ P., LEBEDOVÁ J., PELCLOVÁ D., CHVALOVÁ M., MAREČKOVÁ H., BRABEC M.: Změny buněčných parametrů v indukovaném sputu během specifických bronchoprovokačních testů s profesionálními alergeny (in Czech). *České pracov. lék.* 6(2): 83–88, 2005.
57. KLUSÁČKOVÁ P.: Doktorská dizertační práce "Možnosti časné diagnostiky profesionálního bronchiálního astmatu" (in Czech), 2008.