Arch. Immunol. Ther. Exp. (2013) 61:327–332
DOI 10.1007/s00005-013-0224-3

SHORT COMMUNICATION

Increased cys-Leukotrienes in Exhaled Breath Condensate and Decrease of PNIF after Intranasal Allergen Challenge Support the Recognition of Allergic Rhinitis in Children

Wioletta Zagórska · Katarzyna Grzela · Marek Kulus · Maciej Sobczyński · Tomasz Grzela

Received: 20 July 2012 / Accepted: 25 March 2013 / Published online: 7 April 2013

Abstract Exhaled breath condensate (EBC) contains various mediators of inflammation. Since their concentrations correlate with severity of inflammatory response, EBC assessment allows non-invasive detection of various respiratory tract diseases and enables monitoring of their progression or treatment effectiveness. In this study, authors evaluate the usefulness of cysteinyl leukotrienes (cysLT) measurement in EBC, as non-invasive diagnostic markers of allergic rhinitis in children. It has been found that the assessment of cysLT in EBC, when performed out of the natural allergen exposure, can discriminate between healthy and allergic rhinitis individuals, with sensitivity 87.8 % and specificity 76.4 %, at the threshold level 39.05 pg/ml. The change of peak nasal inspiratory flow (ΔPNIF), measured before and after intranasal allergen challenge allowed recognition of healthy/allergic rhinitis-suffering individuals with sensitivity 76.8 % and specificity 78.6 %, at the threshold level of −3.2 l/min. When ΔPNIF assessment was combined with the measurement of cysLT in EBC, the sensitivity of such diagnostic approach reached 100 % and its specificity increased up to 84.6 %. The proposed algorithm was found to sufficiently discriminate between allergic rhinitis-suffering and healthy children, however, its clinical usefulness especially in young children requires further studies.

Keywords Allergic rhinitis · Cysteinyl leukotrienes · Exhaled breath condensate · Peak nasal inspiratory flow (PNIF)

W. Zagórska · K. Grzela · M. Kulus
Department of Pediatrics, Pneumonology and Allergology, Medical University of Warsaw, Warsaw, Poland

M. Sobczyński
Department of Genomics, Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland

T. Grzela (✉)
Department of Histology and Embryology, Medical University of Warsaw, Chalubinskiego 5, 02-004 Warsaw, Poland
e-mail: tgrzela@ib.amwaw.edu.pl; tomekgrzela@gmail.com

Introduction

Current trends in medical diagnostics and treatment are focused on development of less, or non-invasive methods to detect and monitor various pathologies. The less invasive techniques are associated with minimal risk of complications, thus, increasing safety of medical procedures. Such idea reflects in formerly developed technique of condensation and liquefaction of the exhaled air (Scheideler et al. 1993). The obtained exhaled breath condensate (EBC) contains detectable amounts of various biologically active factors, including pro-inflammatory cytokines and phospholipid-derived mediators, e.g., prostaglandins, thromboxanes, and leukotrienes (Loukides et al. 2011; Rosias et al. 2004). It was proven that concentration of these factors in EBC correlated with intensity of inflammatory reaction, which affected the respiratory tract (Ko et al. 2007). Moreover, it was demonstrated that this method provided data corresponding to those obtained from the bronchoalveolar lavage (BAL) (Antczak et al. 2011; Ono et al. 2008). However, in contrast to BAL, the collection of EBC is non-invasive, risk-free procedure, and could be used even in very young children, without necessity of anesthesia.

Cysteinyl leukotrienes (cysLT) are well-recognized mediators of inflammation with the increasing importance in diagnostic approaches (Antczak et al. 2011; Montuschi and Barnes 2002; Ono et al. 2008). It has been proven that cysLT

levels in BAL fluid and in EBC correlated with severity of inflammatory reaction in respiratory system and with asthma exacerbation (Antczak et al. 2011; Ono et al. 2008).

The early recognition of allergic asthma, especially in young children, may be difficult. Interestingly, significant coincidence of asthma and allergic rhinitis (AR) has been reported by many authors (Becky Kelly et al. 2003; Bousquet et al. 2008). Furthermore, Masuda et al. (2008) have found that almost 77 % of asthmatic patients displayed AR symptoms, which preceded asthma onset for many years. However, the correct identification of AR in young children may also meet some problems, mainly due to high prevalence of nasopharyngeal infections in this group, which may impair the effectiveness of diagnostic procedures. Thus, it would be very useful to find specific diagnostic markers of AR in children. Therefore, authors attempted to analyze the levels of cysLT in EBCs from AR-suffering patients and from healthy children. Then, these data were combined with results of peak nasal inspiratory flow (PNIF), measured before and after intranasal allergen challenge, and estimated with regard to their usefulness as specific indicators of AR.

Materials and Methods

The study involved 83 children (mean age 11.9 ± 4.8, with age range 6–18): 49 children with AR and 34 healthy controls. Patients with AR were further classified into two groups; in addition to basic diagnosis (AR), 20 patients displayed symptoms of episodic asthma (AR + A group), whereas 29 children did not reveal any clinical manifestation of asthma (AR group). The diagnosis criteria were based on indications of *ARIA* Allergic rhinitis and its impact on asthma (Bousquet et al. 2008) and *GINA* Global Initiative for Asthma, Strategy for Asthma Diagnosis and Prevention (updated 2009, available from http://www.ginasthma.org). All patients were in stable condition, during the study period they did not receive inhaled corticosteroids, H_1 receptor antagonists or leukotriene receptor antagonists. All children and their parents gave informed written consent to participate in the study, which was formally approved by the local ethics committee (approval no. KB/93/2008).

According to inclusion criteria, all patients, but none of control group, were monosensitized to grass pollen, and nonreactive to other common seasonal and perennial allergens (pollens of trees, mugwort and plantain, animal hair allergens of dog, cat, hamster and guinea pig, *Dermatophagoides* species and moulds: *Alternaria, Cladosporium, Penicillium*), as was verified by skin prick tests (with mean wheal diameter >3 mm considered as a positive reaction). All patients had specific IgE immunoreactive with timothy, with the serum level above 0.7 kU/l; as assessed by CAP-FEIA Immunoassay (Pharmacia, Germany). Furthermore, routine peripheral blood tests with analysis of leukocytes (total count and populations), spirometry with assessment of FEV_1 using Lung Test 1000 device (MES, Poland), measurements of peak expiratory flow using Portable Flow Meter Mini Wright (HS Clement-Clarke Int, UK) and PNIF using In-Check Nasal device (HS Clement-Clarke Int, UK) were done. EBC was collected using ECoScreen condenser device (Jäger, Germany).

Outside the natural allergen exposure season, in the winter, the patients and healthy controls were subjected to EBC collection and double blind placebo-controlled intranasal allergen provocation associated with PNIF measurement. Patients from each group were randomly divided into two challenge arms. Briefly, after 15–20 min of adaptation to ambient condition, EBC was collected for 15 min, followed by PNIF measurement (baseline). Afterwards, in all individuals the control solution was applied into the nasal cavity, and after 15 min the pre-challenge PNIF measurement was performed. Then, according to the result of randomization, grass pollen suspension, 5,000 BU/ml, (Allergopharma, Germany), or the same volume of placebo solution, was used for the intranasal challenge. After 15 min, the post-challenge PNIF measurement followed by the next 15-min-long EBC collection was done. The PNIF change was calculated according to formula: $\Delta PNIF = PNIF_{after} - PNIF_{before}$. At each quarter of an hour-time point, the patient was asked to respond to short self-assessment questionnaire with the symptom score.

Collected EBC samples were immediately deep frozen and stored at −70 °C, until being used for further analysis. Following the literature and manufacturer's recommendations, the maximal storage time for EBC samples was no longer than 3 months (Montuschi 2009; Ohanian et al. 2010). The Cysteinyl Leukotriene Express Enzyme Immunoassay was performed in duplicates, according to detailed instruction provided by the manufacturer (Cayman Chemical, USA). The sample absorbance was measured using Microplate Reader 550 device (BIO-RAD, USA). The calculation of the cysLT concentration in analyzed condensates (cysLT/EBC) was based on the calibration curve from the standard included in the assay.

Results

According to inclusion/exclusion criteria all children with AR (AR and AR + A groups), but none of control individuals, had a characteristic history of seasonal symptoms with positive skin prick tests for grass pollen and elevated total and specific IgE (Table 1). The reactivity to other common seasonal and perennial allergens was excluded. The presence of acute infection was excluded by normal leukocyte counts

Table 1 The clinical characteristics of study groups

Clinical feature/patient group	Allergic rhinitis (AR)	Allergic rhinitis with asthma (AR + A)	Healthy control
Number of individuals	29	20	34
Sex distribution (female/male)	12/17	4/16	19/15
Age (years)	12.0 ± 4.3	10.9 ± 3.9	12.4 ± 4.9
Body mass (kg)	54.5 ± 23.0	43.8 ± 19.1	50.1 ± 20.3
PEF_{SDS} (l/min)	0.5 ± 0.2	0.6 ± 0.1	0.3 ± 0.2
$FEV1_{SDS}$ (l)	−0.3 ± 0.2	−0.8 ± 0.6	−1.1 ± 0.5
IgE serum level			
Total (kU/l)	199.9 ± 38.2*	269.8 ± 54.7*	33.2 ± 9.8
Specific (kU/l)	26.0 ± 10.1*	20.3 ± 8.4*	0.1 ± 0.2

Mean values ± SD

PEF peak expiratory flow, *SDS* standard deviation score (*Z*-score), value corrected in relation to the age

* Statistically significant, as compared to healthy control group

in all patients and control subjects. The clinical characteristics of analyzed groups and main results of their laboratory and functional tests were shown in Table 1.

The mean PNIF values at baseline (not shown) and before allergen challenge did not differ significantly between all analyzed groups (Fig. 1a). However, in both groups of AR patients, AR and AR + A, the intranasal application of grass pollen suspension resulted in significant decrease of mean PNIF values (mean ΔPNIF = −21.9 l/min, or −27.3 %; and −25.6 l/min, or −31.0 %, respectively). No statistically significant differences in mean PNIF after allergen challenge and in mean ΔPNIF between AR and AR + A groups were observed. In contrast to both AR groups, in healthy controls the application of allergen suspension resulted in significant increase of mean PNIF (ΔPNIF = 11.7 l/min, or 17.8 %) (Fig. 1b). The increased mean PNIF values were also observed in all individuals receiving placebo solution, including AR (mean ΔPNIF = 19.7 l/min, or 21.4 %); AR + A (mean ΔPNIF = 18.8 l/min, or 17.6 %); and control subjects (mean ΔPNIF = 18.5 l/min, or 24.5 %) (Fig. 1a, b). No statistically significant difference in mean PNIF after allergen challenge and ΔPNIF between all placebo-treated groups was observed (Fig. 1b).

The collection of EBCs of patients involved in the study was performed in the winter, outside the natural allergen exposure. Their analysis has shown that baseline concentrations of cysLT/EBC did not differ significantly between AR and AR + A groups (mean values: 51.7 ± 12.5 and 48.8 ± 16.2 pg/ml, respectively). However, in both groups the baseline cysLT/EBC concentrations were statistically significantly higher (with $p < 0.001$, by Mann–Whitney *U* test), when compared to that of healthy controls (mean: 23.2 ± 17.7 pg/ml) (Fig. 2a). Interestingly, in none of study groups, the placebo solution, but also the intranasal allergen application, did not influence significantly mean cysLT/EBC levels, measured within 30 min from exposure (Fig. 2b). No adverse events associated with the entire procedure, especially intranasal allergen challenge or EBC collection, were observed.

The assessment of PNIF change (ΔPNIF), following the intranasal allergen challenge, enabled the discrimination between "healthy" and "AR" individuals. The statistical analysis and the Monte Carlo simulation method (Baurley et al. 2010) established the threshold level for the assay for −3.2 l/min, which provided the sensitivity 76.8 % and specificity 78.6 % (Fig. 3). Regrettably, this method did not allow to distinguish between AR and AR + A individuals.

Using the same modeling method, as mentioned above, the assessment of cysLT concentration in EBC at the threshold level 39.05 pg/ml, enabled the differentiation between "healthy" and "AR" individuals, without necessity of using the intranasal allergen challenge (Fig. 2a). This method revealed the sensitivity 87.8 % and specificity 76.4 %, providing the odds ratio: 3.85. Similarly to the ΔPNIF assessment, the cysLT/EBC measurement alone did not allow to discriminate between AR and AR + A patients.

Interestingly, when cysLT/EBC measurement was combined with ΔPNIF assessment, using both previously mentioned threshold levels, specificity of such diagnostic tool increased to 84.6 %, whereas its sensitivity reached 100 %, with the odds ratio: 24.5 (Fig. 3). Unfortunately, the combined approach, although more effective in "AR"/"healthy" discrimination, also failed to distinguish between patients with (AR + A), or without asthma (AR).

Discussion

The recognition and treatment of AR and asthma may be difficult and arduous for both patient and physician. Some diagnostic procedures may be complicated to conduct, particularly in young children. Therefore, it is necessary to develop a safe and non-invasive method, which would not require close collaboration between patient and medical staff. The intranasal allergen challenge with a self assessment remains the standard diagnostic procedure. However, it may be unfeasible in children of pre-school age. This issue may be resolved by the use of PNIF

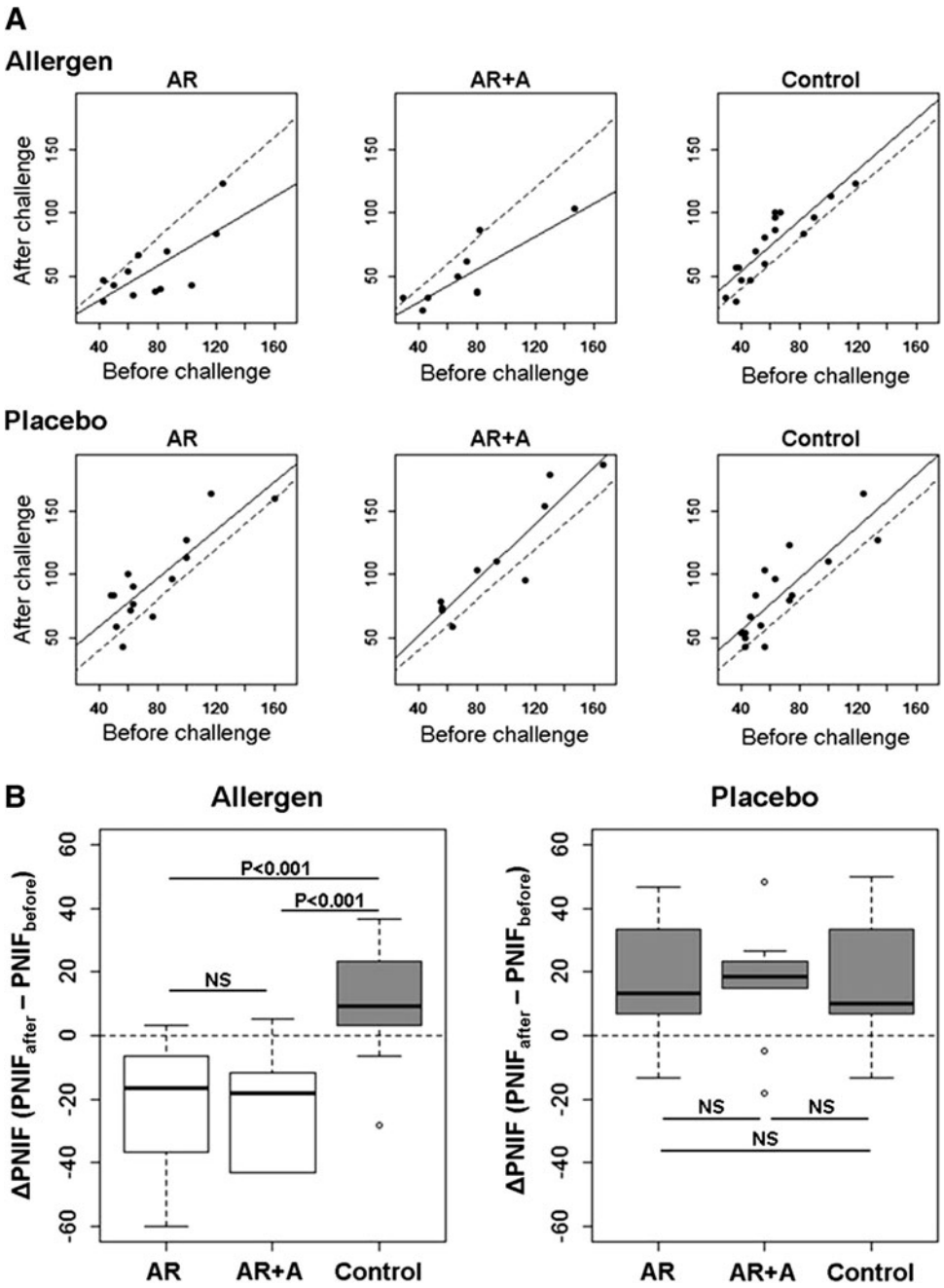

Fig. 1 The results of PNIF measurement (in l/min) before and after intranasal allergen provocation were shown on panel A. Both challenge arms (allergen or placebo) were shown in series of three consecutive graphs. Each *dot* represents one patient from respective group—allergic rhinitis without asthma (AR); allergic rhinitis with asthma (AR + A); and healthy (control) individuals. The *solid lines* represent regression lines calculated for PNIF change after provocation, whereas *dashed lines* correspond to the situation, when PNIF value before and after challenge remains the same ($\alpha = 1$). The mean PNIF changes (ΔPNIF, expressed in l/min) in study groups in respect to provocation mode (allergen or placebo) were shown using *box*-and-*whisker plots* (panel B). The *solid line* in each *box* represents the median value, whereas the *whisker ends* correspond to 9th and 91st percentiles, respectively. *NS* non-significant

measurement. It is a non-invasive method, which reduces significantly the role of subjective estimation of symptoms. Nevertheless, its sensitivity and specificity may be non-satisfactory, since approximately 20–25 % individuals remain with false negative diagnosis. Hence, still there is the necessity to find another diagnostic tool, which alone, or in combination with PNIF assessment would allow the effective recognition of AR. According to numerous reports and our own experience, the EBC may be valuable diagnostic material for this purpose, especially since its collection is safe and easy method even in very young children (Griese et al. 2001; Rosias et al. 2010). The increased concentration of cysLT detected in EBC results from the presence of inflammatory reaction in respiratory system. Interestingly, patients with AR, but without asthma (AR group) displayed elevated cysLT/EBC levels similar to those individuals with asthma (AR + A). This finding may be somehow confusing, since formerly the AR was considered as the local disease, limited to the nasal cavity. However, according to the "united airways" hypothesis, it was postulated recently that initially local reaction, including AR, may

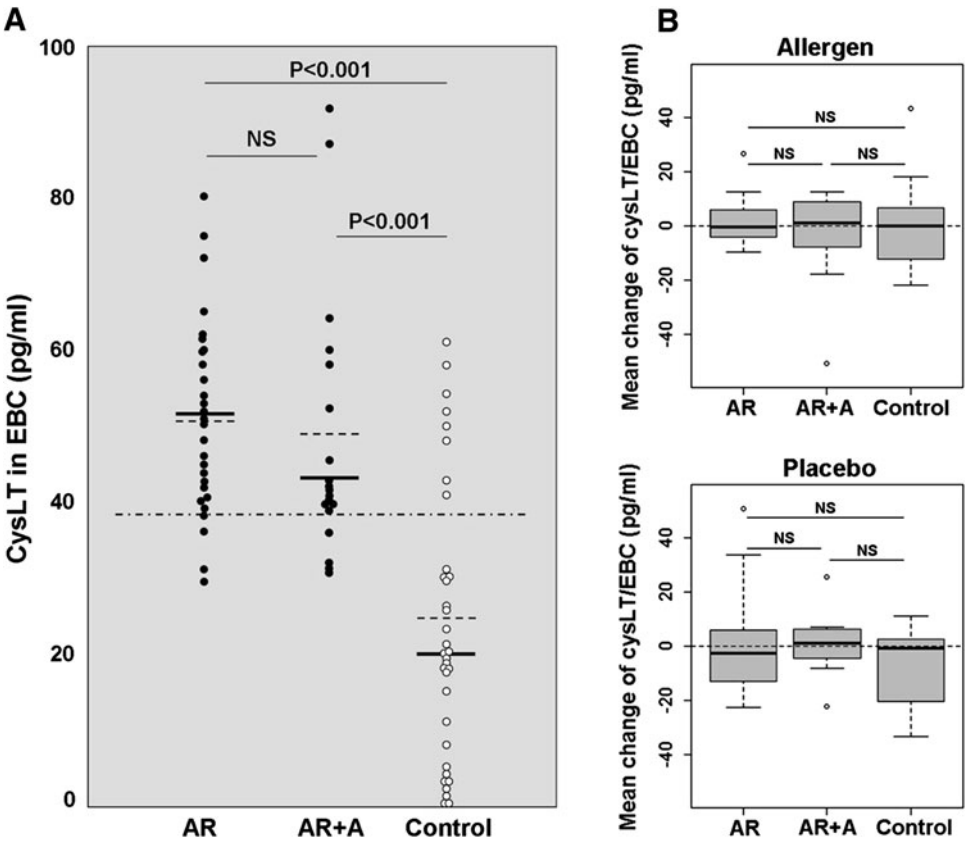

Fig. 2 The concentration (pg/ml) of cysteinyl leukotrienes (cysLT) in exhaled breath condensates (EBC) at baseline (panel A). Each *dot* represents one individual; *filled black dots* show children with allergic rhinitis alone (AR) and patients with allergic rhinitis and asthma (AR + A); *white dots* correspond to healthy controls. The mean values of cysLT concentration in each group were shown as *dashed lines*, whereas median values were shown as *solid lines*. *Dash/dot line* represents the threshold level for the "allergic rhinitis/healthy" discrimination. The mean cysLT/EBC changes (expressed in pg/ml) in study groups in respect to provocation mode (allergen or placebo) were shown using *box*-and-*whisker plots* (panel B). The *solid line* in each box represents the median value, whereas the *whisker ends* correspond to 9th and 91st percentiles, respectively. *NS* non-significant

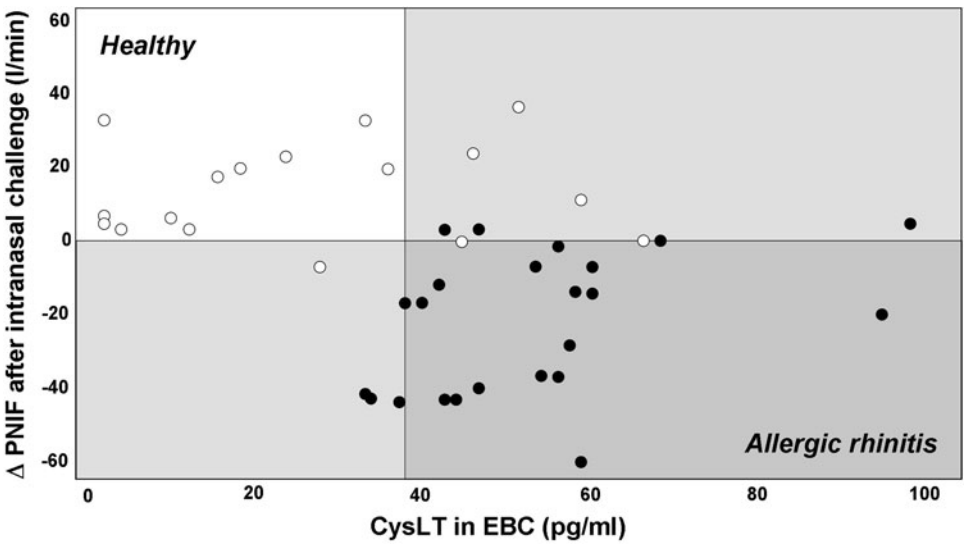

Fig. 3 The threshold levels for the "allergic rhinitis/healthy" discrimination. The *horizontal line* represents "allergic/healthy" discrimination point for PNIF change (ΔPNIF) in response to intranasal allergen challenge. The *vertical line* corresponds to the "allergic rhinitis/healthy" discrimination point for the cysLT concentration in EBC. Each *dot* represents single individual; *filled black dots* show children from both AR and AR + A groups; *white dots* correspond to healthy controls. This two-parameter assessment may help to identify the patient as "healthy" (white quadrant), "possibly allergic rhinitis" (*two light grey quadrants*) or "definitely allergic rhinitis" (*dark grey quadrant*)

also involve the other segments of respiratory tract, thus influencing the EBC composition (Bonay et al. 2006; Inal et al. 2008). Our finding seems to support this hypothesis.

On the other hand, this hypothesis may be discordant with the observation regarding the similar cysLT/EBC concentrations before and after the intranasal allergen

challenge. One can expect increased cysLT/EBC, even after local allergen provocation (Beeh et al. 2003; Bonay et al. 2006; Inal et al. 2008). Therefore, this issue needs further elucidation.

The interesting observation regarding the intranasal allergen challenge was done in placebo receiving patients. The application of placebo solution resulted in increased PNIF values in all groups. It is plausible that the observed response could be explained by the "washing" effect of neutral placebo solution. Moreover, the allergen suspension might possibly reveal similar effect also in healthy children.

The most surprising result in our study was the lack of significant differences in tested variables between AR individuals with (AR + A), or without asthma (AR). Possibly, the similar results in cysLT/EBC and PNIF changes of AR and AR + A individuals may reflect similar intensity of inflammatory reaction in both groups. The question, whether lack of differences between AR and AR + A patients results from subclinical asthma in AR patients, or rather depends on well-controlled asthma in AR + A individuals, remains unanswered.

As shown above, the proposed method allows a fast discrimination between healthy and AR-suffering patients. The main advantage of this non-invasive diagnostic tool is its safety and low risk of adverse events. It allows the recognition of seasonal allergy independently. Moreover, possibly, this approach may be an attractive solution for monitoring the effectiveness of allergen immunotherapy, or treatment with intranasal steroids. The main inconvenience of this system seems to be a requirement of an access to deep freezer and short storage time allowed for cysLT samples.

It is noteworthy that the proposed algorithm, although sufficient to discriminate between healthy and AR-suffering children, did not enable to distinguish patients with, or without asthma. Possibly, the cysLT/EBC measurement is not sensitive enough to detect any differences (if present) in intensity of respiratory tract-affecting inflammatory reaction between AR-suffering children from AR and AR + A groups. However, on the other hand, it is also plausible that the similar cysLT/EBC levels and PNIF responses observed in both asthmatic and non-asthmatic individuals may correspond to the presence of subclinical inflammation also in those children, who were initially diagnosed as non-asthmatic. Regardless of fact that this issue still requires further elucidation, the measurement of cysLT/EBC could be considered as a valuable method for fast and effective screening of AR even in pre-school young children (Griese et al. 2001; Rosias et al. 2010).

References

Antczak A, Piotrowski W, Marczak J et al (2011) Correlation between eicosanoids in bronchoalveolar lavage fluid and in exhaled breath condensate. Dis Markers 30:213–220

Baurley JW, Conti DV, Gauderman WJ et al (2010) Discovery of complex pathways from observational data. Stat Med 29:1998–2011

Becky Kelly EA, Busse WW et al (2003) A comparison of the airway response to segmental antigen bronchoprovocation in atopic asthma and allergic rhinitis. J Allergy Clin Immunol 111:79–86

Beeh KM, Beier J, Kornmann O et al (2003) A single nasal allergen challenge increases induced sputum inflammatory markers in non-asthmatic subjects with seasonal allergic rhinitis: correlation with plasma interleukin-5. Clin Exp Allergy 33:475–482

Bonay M, Neukirch C, Grandsaigne M et al (2006) Changes in airway inflammation following nasal allergic challenge in patients with seasonal rhinitis. Allergy 61:111–118

Bousquet J, Khaltaev N, Cruz AA et al (2008) Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA^{2}LEN and AllerGen). Allergy 63(Suppl 86):8–160

Griese M, Latzin P, Beck J (2001) A noninvasive method to collect nasally exhaled air condensate in humans of all ages. Eur J Clin Invest 31:915–920

Inal A, Kendirli SG, Yilmaz M et al (2008) Indices of lower airway inflammation in children monosensitized to house dust mite after nasal allergen challenge. Allergy 63:1345–1351

Ko FW, Leung TF, Hui DS (2007) Are exhaled breath condensates useful in monitoring asthma? Curr Allergy Asthma Rep 7:65–71

Loukides S, Kontogianni K, Hillas G et al (2011) Exhaled breath condensate in asthma: from bench to bedside. Curr Med Chem 18:1432–1443

Masuda S, Fujisawa T, Katsumata H et al (2008) High prevalence and young onset of allergic rhinitis in children with bronchial asthma. Pediatr Allergy Immunol 19:517–522

Montuschi P (2009) LC/MS/MS analysis of leukotriene B4 and other eicosanoids in exhaled breath condensate for assessing lung inflammation. J Chromatogr B Analyt Technol Biomed Life Sci 877:1272–1280

Montuschi P, Barnes PJ (2002) Exhaled leukotrienes and prostaglandins in asthma. J Allergy Clin Immunol 109:615–620

Ohanian AS, Zimmerman J, Debley JS (2010) Effects of sample processing, time and storage condition on cysteinyl leukotrienes in exhaled breath condensate. J Breath Res 4:046002

Ono E, Mita H, Taniguchi M et al (2008) Comparison of cysteinyl leukotriene concentrations between exhaled breath condensate and bronchoalveolar lavage fluid. Clin Exp Allergy 38:1866–1874

Rosias PP, Dompeling E, Dentener MA et al (2004) Childhood asthma: exhaled markers of airway inflammation, asthma control score, and lung function tests. Pediatr Pulmonol 38:107–114

Rosias PP, Robroeks CM, van de Kant KD et al (2010) Feasibility of a new method to collect exhaled breath condensate in pre-school children. Pediatr Allergy Immunol 21(2 Pt):e235–e244

Scheideler L, Manke HG, Schwulera U et al (1993) Detection of non-volatile macromolecules in breath. A possible diagnostic tool? Am Rev Respir Dis 148:778–784