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Association of Asthma Symptoms and Exacerbation with Inflammatory Biomarkers

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Abstract

Objective: To investigate a relationship of asthma symptoms and exacerbations with systemic (high-sensitivity C-reactive protein (hs-CRP), eosinophilic cationic protein (ECP), leukocytes) and local (exhaled NO (FENO), pH and urates in exhaled breath condensate (EBC) and exhaled breath temperature (EBT)) inflammatory biomarkers (BMs) in asthmatic children.

Methods: This cross-sectional study comprised 93 consecutive asthmatic patients (age 6-18 years, 22 girls) with mild intermittent asthma ([IA], N=44) and mild to moderate persistent asthma ([PA], N=49). Medical history (asthma symptoms and exacerbation), pulmonary function, FENO, EBT, samples of exhaled breath and peripheral blood were collected.

Results: Local BMs (EBC urates and EBT) showed stronger correlation with asthma symptoms then systemic BMs (hs-CRP, blood count with differencials) (r=0.26-0.68, r=0.06-0.32, p<0.05; respectively). Single measurements of inflammatory BMs are not good predictors for future asthma exacerbation (binary logistic regression; χ^2=13.9; df=11; p=0.238).

Conclusion: Study of combination of various exhaled breath and exhaled breath condensate BMs should continue, especially in longitudinal studies with repeated measurements of BMs.

Keywords: Asthma; Biomarkers; Exhaled breath condensate; Exhaled breath temperature; High-sensitive C-reactive protein

Introduction

Asthma is a heterogeneous disease of the airways involving chronic airway inflammation, increased airway responsiveness and tissue remodelling. Evaluation of asthma severity and control includes two domains namely impairment, which includes an evaluation of the frequency and intensity of symptoms; and risk, which includes an assessment of the likelihood of asthma exacerbation [1].

Biomarkers (BMs) are measurable biologic indicators of a particular disease state that can be useful in objective evaluation of normal or pathogenic process as, well as a measure of the efficacy of therapeutic interventions [2]. Hence, BMs can be diagnostic, prognostic, predictive or pharmacodynamic. Diagnostic BMs enable diagnosis of the disease, prognostic BMs provide information about an outcome of pathogenic process regardless of the therapy, predictive BMs determine an outcome of pathogenic process after the specific therapy [3], and pharmacodynamic BMs indicate the efficacy of therapeutic intervention on its target [4].

Hs-CRP (high-sensitive C-reactive protein) has already been confirmed as a systemic inflammatory BM in patients with asthma [5]. Recently, the great interest has been directed to exhaled breath [6] and different compounds in exhaled breath condensate (EBC) [7], to determine local BMs involved in the respiratory system inflammation. Exhaled breath temperature, EBT, may be used as a useful parameter of inflammation in the airways [8].

However, no specific studies have been conducted in asthma to establish a causal relationship between bronchial and systemic inflammation in asthma and asthma symptoms. Recent studies evaluate different inflammatory markers from sputum and exhaled breath with the severity and/or control of asthma (sometimes evaluating symptoms score as primary or secondary variable), ensuring to assess indirectly relationship between inflammation and asthma symthoms. However, there are mainly cross-sectional or longitudinal studies analysing inflammatory cells in the sputum, in particular eosinophils and the fraction of exhaled NO (FENO). Other studies have evaluated the general effect of a treatment or therapeutic strategy. A few studies have investigated tissue inflammation with bronchial biopsies, and more recently, exhaled breath condensates and their relation to symptoms [9].

We hypothesized that asthma symptoms correlate with biomarkers (BMs) of inflammation in asthmatics. The aim of study was (1) to investigate a relationship of systemic inflammatory BMs (hs-CRP, leukocytes) and local inflammatory BMs (FENO, EBT, EBC urates and pH) with asthma symptoms (cough, wheezing, chest tightness, heavy breathing, shortness of breath, need to clear a throat, daytime and nocturnal symptoms, need for reliever), and (2) to investigate the value of inflammatory BMs as predictors of future asthma exacerbation.

Materials and Methods

Subjects

This cross-sectional study included ninety-three consecutive children and adolescents (aged 6-18 years (± SD = 12 ± 3 years), of which 22 girls (23.7%)) with mild intermittent asthma (IA, N=44) and mild to moderate persistent asthma (PA, N=49) [1] between June 2011 and December 2012 from Outpatient clinic Srebrnjak Children's Hospital. Table 1 shows characteristics of studied subjects. Asthma was diagnosed based on ATS (American Thoracic Society) and GINA (Global Initiative for Asthma) guidelines [10,11] at least a year before the inclusion visit. Atopic status was determined by skin prick tests and/or increased level of specific IgE at the time when asthma was diagnosed to the patient. Skin prick tests were performed according to the European Academy of Allergology and Clinical Immunology (EAACI) guidelines [12] using a standard series of inhalatory allergens together with positive (histamine) and negative control. The tests have been considered positive if mean wheal diameter was ≥ 3 mm compared with negative control. The concentration of total IgE was determined by microparticle enzyme immunoassay (MEIA) method and reagents (Abbott, Abbott Park, IL, USA) using mouse monoclonal anti-IgE antibodies. Measurement of fluorescence was done using an IMx autoanalyzer (Abbott, Abbott Park, IL, USA). The concentration of allergen-specific IgE was measured by the UniCAP method (Pharmacia, Uppsala, Sweden) on a selective UniCAP 100 autoanalyzer (Pharmacia-LKBKabi,Uppsala, Sweden). At the time of evaluation 72 (77.4%) children were, according to seasonality, exposed to aeroallergens to which they were sensitized. Patients were on the stable dose of their regular asthma treatment, with exception of prn SABA {lat. "pro re nata - as needed, short-acting β2-agonist, (Table 1)}, during the time from preceding clinical visit (1-3 months ago, depending on patients' level of asthma control). Patients were not taking vitamin supplementation therapy, or N-acetylcysteine, or other antioxidants during the preceding month.

	(N=93)	IA (N=44)	PA (N=49)
Gender, male, No (%)	71 (76.3)	35 (79.5)	36 (73.5)
Age, mean (SD) [range], year	12 (3) 6-18	11.9 (3.5) 6-18	11.8 (3.19) 6-18
Atopic status, No (%)*			
No sensitization	17 (18.3)	9 (20.45)	8 (16.3)
Sensitized	76 (81.7)	35 (79.5)	41 (83.7)
Allergen exposure. No (%)	72 (77.4)	34 (77.3)	38 (77.6)
Current asthma treatment, No (%)			
Only SABA prn	14 (15.1)	5 (11.4)	9 (18.4)
ICS	28 (30.1)	8 (18.2)	20 (40.8)
LTRA	7 (7.5)	4 (9.1)	3 (6,1)
ICS + LABA	40 (43)	24 (54.4)	16 (32.6)
ICS + LTRA	3 (3.2)	2 (4.5)	1 (2)
ICS + LABA + LTRA	1 (1.1)	1 (2.3)	0 (0)
SABA used**	44 (47.3)	36 (81.8)	8 (16.3)
Past exacerbation, No (%)***	44 (44.1)	36 (81.8)	5 (10.2)
Future exacerbation, No (%)****	15 (16.1)	12 (27.3)	3 (6.1)
Lung function measurements			
FEV1, mean (SD), % of predicted *****	92, 5 (14.3)	87, 8 (16.1)	96, 7 (11)
FEV1/FVC, mean (SD), % of predicted ******	81, 6 (7.8)	79, 2 (9.3)	83, 9 (5.5)
MEF50, mean (SD), % of predicted *******	82, 6 (23.2)	75, 4 (25.3)	89 (19.4)

Note: IA: mild intermittent asthma; PA: mild to moderate persistent asthma; SD: standard deviation; BMI: body mass index; IQR: interquartile range; prn: as needed; SABA: short acting β2-agonists; ICS: inhaled corticosteroids; LTRA: leukotrien antagonists; LABA: long acting β2-agonists; FEV1: forced expiratory volume in one second; MEF50: maximal expiratory flow at 50% respiratory function.

*atopic status - determined by skin prick tests and/or increased level of specific IgE at the time when asthma was diagnosed to the patient. ** χ^2=14.64, df=1, p<0.0001; *** future exacerbation - future asthma exacerbation in next 6 months from the initial visit reported by the patient χ^2=21.26, df=1, p<0.0001; ****χ^2=5.53, df=1, p=0.0173; (Fisher exact test).

***** p=0.002 t=-3.171; ******; p=0.004, t=-2.987; ****** p=0.004, t=-2.922 (Student t-test)

Table 1: Characteristics and outcames of studied subjects (N=93). Characteristics and outcames of mild intermittent asthma (IA, N=44) and mild to moderate persistent asthma (PA, N=49).

Diagnostic work-up was performed according to standardized in-house procedure (according to GINA guidelines), and in line with ethical principles (approved by Hospital Review Board) and Declaration on Human Rights from Helsinki 1975 and Tokyo amendments 2004 - 2008 [13]. All subjects and/or parents consented for the study.

Exclusion criteria included subjects with obesity (body mass index (BMI) over the 85th percentile for age)), diabetes mellitus, cancer, systemic inflammatory disorders, subjects with serum CRP levels of >2.5 mg/L, and subjects with a respiratory tract infection during a preceding month. Gastroesophageal reflux disease was excluded in asymptomatic patients by anamnestic and clinical data or by 24 hours pH monitoring study in asthmatics with signs and symptoms suggestive for gastroesophageal reflux.

Methods

Medical history (asthma symptoms, adherence to asthma medication plan, prn use of SABA, health resource utilization) was assessed together with physical examination, spirometry, FENO (Fractional Exhaled Nitric Oxide), EBT (Exhaled Breath Temperature), blood samples and exhaled breath condensate data.

The frequency of asthma symptoms (chest tightness, heavy breathing, wheezing, cough, need to clear a throat, difficulty breathing out, wake in a.m. with symptoms, shortness of breath, woken at night by asthma, lack of a good night's sleep, fighting for air) was assessed according to 7-point Likert scale (7=not impaired at all - 1=severely impaired). Blood sampling was done upon clinical examination between 8.00 a.m. and 12.00 a.m. Following parameters were analyzed in peripheral blood specimen: white blood cell count (WBC, leukocytes) and differential cell counts, hs-CRP and ECP. Venous blood samples were centrifuged at 1300 g for 10 minutes at room temperature.

Leukocytes and differential cell counts were determined using a Sysmex XT-1800i flow cytometer (Sysmex, Kobe Hyogo, Japan). The concentration of ECP wwas determined by the fluorescence enzyme immunoassay (FEIA) (ImmunoCAP, LKB, Uppsala, Sweden) method on a selective UniCAP 100 auto-analyzer (LKB, Uppsala, Sweden) [14]. The concentration of hs-CRP was determined within 1 hour by latex-enhanced immunoturbidimetric method [15] on a Beckman Coulter AU 400 automated biochemistry analyzer (Beckman Coulter, Tokyo, Japan), using Beckman Coulter reagents (Beckman Coulter Life and Material Science Europe, Hamburg, Germany).

FENO was measured with the single exhalation method at 50 mL/s during 10 seconds using a NiOX analyzer (Aerocrine, Stockholm, Sweden) according to current European Respiratory Society and ATS (ERS/ATS) recommendations [16].

Lung function measurements: forced expiratory volume in one second, FEV1, forced vital capacity, FVC, and FEV1/FVC, respectively, maximal expiratory flow at 50% respiratory function, MEF50, and peak expiratory flow, PEF post bronchodilator FEV1, post bronchodilator FEV1/FVC, post bronchodilator FVC were measured using a computerized pneumotach (Ganzhorn, Germany) in accordance with ATS guidelines [17] and presented as percentage of predicted values according to Quanjer [18].

EBT were measured using an X-halo Breath Thermometer (Delmedica, Singapore) according to Popov et al. [19].

EBC samples were obtained between 7:00 and 9:00 am and collected according to the ATS/ERS Task Force recommendation [20] using an EcoScreen condenser (Erich Jaeger GmbH, Oechberg, Germany). Samples were deaerated (CO_2 elimination, i.e., gas standardization) using argon (350 mL/min for 10 min). None of the EBC samples showed detectable α-amylase catalytic activity (detection limit 7 U/L). Measurements of pH in EBC were performed up to 5 min after argon deaerating using a blood gas analyzer (Ecosys, Eschweiler, Germany). Urates measurements were performed up to 10 minutes after EBC collection by an enzymatic color test on a Beckman Coulter AU 400 selective auto-analyzer (Beckman Coulter, Tokyo, Japan). The detection limit for urates concentration was 5 μmol/L (our validation data for analytical sensitivity).

The occurrence as well as the number of exacerbations after the initial visit was questioned at the second visit after 6 month.

Statistical analysis

Data storage and processing for statistical analysis was performed using Microsoft Excel 2013 (Microsoft, USA). Continuous variables were described as mean and standard deviation (± SD) if they had normal distribution, or median and interquartile range (M (IQR)) if not. Blood cell counts were adjusted for age and expressed as Z-values for both absolute (A) and relative (R) counts. Comparisons between groups were made using a Student's t test for normally distributed variables, Mann Whitney test for non-normally distributed variables, and Chi-square test or Fisher exact test for categorical variables. Associations were analyzed using regression analysis (univariate and multivariate models). The multivariate models were modeled using backward stepward analysis as the most conservative approach because of the small sample size (age was always used as a covariate in the models). For categorical variables logistic regression analysis was used. An odds ratio (OR) was used to measure an association between inflammatory biomarkers and asthma symptoms and exacerbations with confidence interval (CI) 95% and p-value.

The data was analyzed using STATISTICA version 10 (StatSoft, Inc. Tulsa, OK) and SPSS (version 15.1; SPSS, Chicago). Statistical significance was set to $p<0.05$ for all tests.

Results

Data on all outcomes for all 93 asthmatic patients are presented in Table 2. Statistically significant differences between groups (IA vs. PA) were found for following BMs: monocytes, basophils, hs-CRP, EBC urates and EBT ($p<0.05$ for all; Table 2).

	(N=93)	IA (N=44)	PA (N=49)	Statistics
WBC, median (IQR), $\times10^9$/L†	6, 7 (2.23)	6, 95 (2.05)	6, 5 (2.43)	Z=1.531, p=0.126
Neutrophils, median (IQR), $\times10^9$/L†	3, 17 (1.56)	3, 245 (1.305)	3, 08 (1.59)	Z=0.854, p=0.393 Z=-0.354, p=0.723
Lymphocytes, mean (SD), $\times10^9$/L†	2, 46 (0.68)	2, 565 (0.73)	2, 4 (0.76)	t=0.629, p=0.531 t=-0.260, p=0.796
Monocytes, median (IQR), $\times10^9$/L†	0, 58 (0.2)	0, 655 (0.205)	0, 55 (0.12)	**Z=2.262, p=0.024** Z=0.885, p=0.376
Eosinophils, median (IQR), $\times10^9$/L†	0, 31 (0.28)	0, 33 (0.33)	0, 31 (0.23)	Z=1.181, p=0.237 Z=-0.846, p=0.397
Basophils,	0, 03 (0.02)	0, 03 (0.02)	0,03 (0.03)	**Z=-1.901, p=0.057**

median (IQR), $\times 10^9$/L†				**Z=-2.093, p=0.036**
hs-CRP, median (IQR), mg/L	0, 4 (0.62)	0, 65 (0.76)	0, 29 (0.39)	**Z=2,38, p=0.017**
ECP, median (IQR), ng/mL	16 (13.2)	17, 1 (14.1)	15, 7 (12.5)	Z=0.554, p=0.513
FENO, median (IQR), ppb	21 (23)	22 (13)	21 (23)	Z=0.904, p=0.366
EBC urates, median (IQR), μmol/L	28, 9 (36)	9 (5.5)	45 (29)	**Z=-6.41, p<0.0001**
EBC pH, median (IQR)	8, 27 (0.21)	7, 252 (0.21)	7, 31 (0.216)	Z=-1.885, p=0.06
EBT, mean (SD), °C	34, 1 (0.77)	34, 4 (0.78)	33, 8 (0.66)	**t= 4.0457, p=0.0001**

Note: IA= mild intermittent asthma; PA= mild to moderate persistent asthma; SD – standard deviation; IQR – interquartile range; prn – as needed; SABA – short acting beta-agonists; ICS – inhaled corticosteroids; LTRA – leukotrien antagonists; LABA – long acting beta-agonists; hs-CRP - high sensitivity CRP; ECP – eosinophilic cation protein; FENO – exhaled NO; EBC exhaled breath condensate; EBT – exhaled breath temperature.

Group comparisons were done using Student's t-test (t-value) and Mann Whitney test (Z-value).

†Group comparisons were done using Student's t-test and Mann Whitney test for Z-scores of absolute (A) and relative (R) WBC and differential counts.

Table 2: Inflammatory biomarkers (BMs: FENO, EBC parameters, EBT, WBC, ECP, and hs-CRP) in study studied subjects (N=93). Comparations between mild intermittent asthma (IA) and mild to moderate persistent asthma (PA).

Univariate regression analysis confirmed positive correlation between EBC urates and EBC pH and frequency of symptoms of asthma (Table 3).

	Biomarker				
Symptom	**hs-CRP**	**FENO**	**EBC urates**	**EBC pH**	**EBT**
Heavy breathing	**r=-0.253, p<0.05**	**r=0.221, p<0.05**	**r=0.586, p<0.05**	**r=0.218, p<0.05**	**r=-0.309, p<0.05**
Wheezing	**r=-0.295, p<0.05**	p>0.05	**r=0.682, p<0.05**	**r=0.226, p<0.05**	**r=-0.357, p<0.05**
Cough	**r=-0.296, p<0.05**	p>0.05	**r=0.697, p<0.05**	**r=0.215, p<0.05**	**r=-0.319, p<0.05**
Chest tightness	**r=-0.208, p<0.05**	p>0.05	**r=0.636, p<0.05**	**r=0.242, p<0.05**	**r=-0.464, p<0.05**
Need to clear a throat	**r=-0.298, p<0.05)**	p>0.05	**r=0.636, p<0.05**	**r=0.256, p<0.05**	**r=-0.300, p<0.05**
Difficulty breathing out	**r=-0.205, p<0.05**	p>0.05	**r=0.599, p<0.05**	**r=0.288, p<0.05**	**r=-0.305, p<0.05**
Wake in a.m. with symptoms	**r=-0.211, p<0.05**	p>0.05	**r=0.479, p<0.05**	**r=0.266, p<0.05**	**r=-0.261, p<0.05**
Shortness of breath	**r=-0.245, p<0.05**	p>0.05	**r=0.582, p<0.05**	**r=0.260, p<0.05**	**r=-0.357, p<0.05**
Woken at night by asthma	**r=-0.267, p<0.05**	p>0.05	**r=0.597, p<0.05**	**r=0.212, p<0.05**	**r=-0.340, p<0.05**
Lack of a good night's sleep	**r=-0.261, p<0.05**	p>0.05	**r=0.597, p<0.05**	**r=0.242, p<0.05**	**r=-0.330, p<0.05**
Fighting for air	**r=-0.221, p<0.05**	p>0.05	**r=0.595, p<0.05**	**r=0.298, p<0.05**	**r=-0.286, p<0.05**

Statistically significant correlations are marked in bold letters.

Table 3: Correlations between biomarkers and symptoms in study studied patients (univariate regression analysis).

Serum hs-CRP and EBT showed negative correlation with frequency of asthma symthoms. A-monocyte Z-score significantly negatively correlated with frequency of asthma symptoms (shortness of breath and woken at night by asthma; r=-0.308, r=-0.326, p<0.05 for

all; respectively). Statistically significant moderate correlation (i.e., r>0.500; p<0.05, Figure 1) was confirmed only between EBC urates and frequency of symptoms. In addition, weak correlation was confirmed between EBT values and chest tightness (r= -0.464, p<0.05, Figure 2).

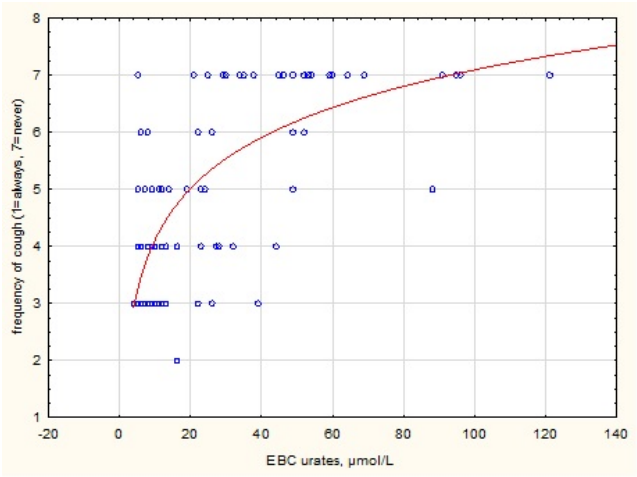

Figure 1: Scatter diagram for frequency of cough vs. urates from exhaled breath condensate (EBC urates) (r=0.697, p<0.05).

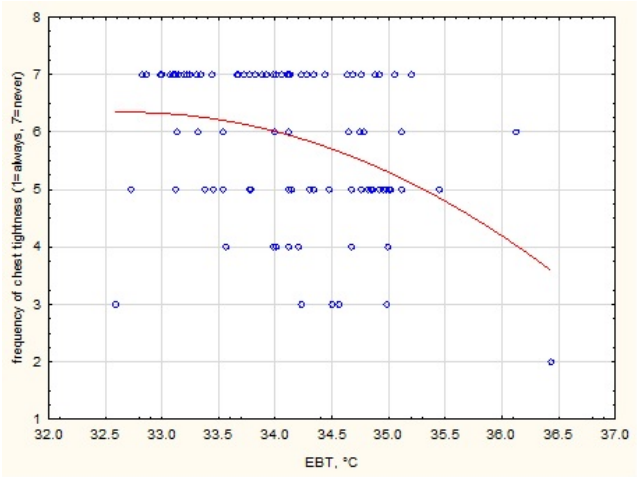

Figure 2: Scatter diagram for frequency correlation of frequency of chest tightness and exhaled breath temperature (EBT) (r=-0.464, p<0.05).

Multiple regression analysis for FENO has shown an independent positive correlation with frequency of cough, shortness of breath and lack of a good night's sleep, and negative correlation with frequency of wake in a.m. with symptoms, (p<0.04 for all; r^2=0.362 for the model, p<0.00015, multiple regression analysis). Multiple regression analysis for EBC urates has shown an independent positive correlation with frequency of cough (p=0.013; r^2=0.497 for the model, p<0.001, multiple regression analysis). Multiple regression analysis for EBT has shown an independent negative correlation with frequency chest tightness (p=0.002; r^2=0.306 for the model, p<0.002, multiple regression analysis).

While increased FENO levels were significantly associated with heavy breathing (OR>1: OR, 3.4133; 95% CI, 1.1552-10.0857; p=0.026), neither EBT, nor EBC urates have shown statistically signifficant association (i.e., OR<1): EBT>34.4°C were associated with the chest tightness with an OR of 0.2917 (95% CI, 0.1044-0.8146; p=0.0187). EBT urates >29 µmol/L were associated with the cough with an OR of 0.1213 (95% CI, 0.03491-0.4211; p=0.0009), wheezing episodes with an OR of 0.1415 (95% CI, 0.03133-0.6388; p=0.011) and a need to clear a throat with an OR of 0.08321 (95% CI, 0.01892-0.366; p=0.001. These results reveal the risk of heavy breathing in patients with increased values of FENO.

EBC urates <25 µmol/L were significantly associated with future asthma exacerbation in next 6 months OR 0.0989 (95% CI, 0.01248-0.7839; p=0.029).

Association between asthma exacerbation and BMs was analyzed using binary logistic regression with asthma exacerbation as dependent variable (0=no; 1=yes) and BMs as independent variables (predictors). Model that included all of local and systemic BMs (χ^2=13.9; df=11; p=0.238) explained between 12.6 and 20.5% variances (Table 4).

	OR (95% CI)	p-value
EBC pH	0.07 (0.0 – 1.99)	0.118
EBC urates	0.97 (0.94 – 1.0)	0.075
EBT	0.51 (0.2 – 1.31)	0.163
ECP	1.00 (0.96 – 1.03)	0.872
FENO	1.01 (0.99 – 1.04)	0.347
hs-CRP	2.05 (0.57 – 7.30)	0.269
WBC	6.78 (0.0 – 2593484)	0.770
Neutrophils	0.18 (0.0 – 76512)	0.798
Lymphocytes	0.16 (0.0 – 63298)	0.783
Monocytes	0.18 (0.0 – 103183)	0.802
Eosinophils	0.55 (0.0 – 351551)	0.929

Note: OR - odds ratio; CI - confidence interval; hs-CRP - high sensitivity CRP; ECP – eosinophilic cation protein; FENO – exhaled NO; EBC exhaled breath condensate; EBT – exhaled breath temperature.

Association between asthma exacerbation and BMs was analyzed using binary logistic regression with asthma exacerbation as dependent variable (0=no; 1=yes) and BMs as independent variables (predictors). Model that included all of local and systemic BMs (χ^2=13.9; df=11; p=0.238) explained between 12.6 and 20.5% variances.

Table 4: Association between biomarkers and future asthma exacerbation in study studied patients (binary logistic regression).

Neither of analyzed BMs was confirmed as predictor for future asthma exacerbation. However, very high variability in WBC with differentials was noticed.

Discussion

The study revealed asthmatic symptoms (i.e., heavy breathing, wheezing, cough, chest tightness, need to clear a throat, difficulty breathing out, wake in a.m. with symptoms, shortness of breath, woken at night by asthma, lack of a good night's sleep and fighting for air) to be in statistically significant positive moderate correlation only with urates in EBC. Chest tightness has shown to be weakly associated with increased EBT levels, and FENO with heavy breathing, i.e., higher FENO imply higher risk of heavy breathing. Neither of these BMs was confirmed as predictor for future asthma exacerbation.

Asthma symptoms constitute essential elements for asthma control and therefore it seems important to evaluate true relation between inflammation and asthma symptoms. Airway inflammation in asthmatics is usually monitoring with exhaled NO and certain number of studies indicates a relation between FENO and asthma symptoms or symptoms score [9]. In our study, FENO weakly positively correlated with heavy breathing. However, a possible effect of anti-inflammatory treatment on FENO values should be consider in interpretation of our results.

Recent studies proposed an EBT as a new noninvasive biomarker of local inflammation in asthma. It is increased in persistent asthmatics and correlate with other markers of local inflammation (FENO and FEV1) [21,22]. However, we found a low to moderate correlation between frequency of asthma symptoms and local inflammation presented with EBT. EBC urates are proposed as a new non-invasive biomarker of altered oxidative-antioxidative balance in exhaled breath in asthmatics [23]. They are decreased in uncontrolled asthmatics [24] possible due to their consumption during increased oxidative stress in uncontrolled asthma. For the first time, we reported a modarete to strong correlation between frequency of asthma symptoms and EBC urates. In fact, frequent asthma symptoms were associated with lower concentration of EBC urates. Koskela et al. reported a relationship between high 8-isoprostane (marker of oxidative stress) in asthmatics with severe cough response to hyperpnoea, low Leicester Cough Questionnaire values (indicating severe subjective cough), and usage of combination asthma drugs [25]. It was hypothesized that reactive oxygen species sensitize/activate sensory C-fibers which are capable to induce cough. This observation is in accordance with our results supporting hypothesis of oxidative-antioxidative inbalance in asthma symptoms pathogenesis. It is of utmost importance to reveal a biomarker of future risk due to insufficiency of traditional tools in assessment of the likelihood of asthma exacerbation. Namely, the limitations of lung function measurements have been seen in Childhood Asthma Management Program study where at baseline mean FEV1 % predicted was more than 93% among this pediatric population, and yet approximately one third of the children had asthma exacerbation 6 months after enrollment [26]. In accordance with that result, in our study, 53.3% of patients with asthma exacerbation in future had FEV1 % predicted >90%. According to Wu et al. predictors for severe asthma exacerbations are: younger age, history of hospitalization or ED visit in the prior year, ≥ 3 days use of oral corticosteroids in the prior 3 months, lower FEV(1)/FVC ratio and a lower natural logarithm of provocative concentration of methacholine producing a 20% decline in FEV1 and higher logarithm to the base 10 eosinophil count [27]. The strongest predictor of future asthma exacerbation in children is past asthma exacerbation [28,29]. Asthma symthoms are poor predictors for future asthma exacerbation [30]. In another study, symptom scores on validated questionnaires were not significantly worse in children who developed an exacerbation [31]. Recently, Robroeks et al. reported that prediction of asthma exacerbations in children is possible by profiles of exhaled volatile organic compounds (VOCs), a new non-invasive inflammatory biomarkers [32]. FENO and lung function were not predictive for exacerbations, as in the present study. The results of presents study for the first time have demostrated a significant association EBS urates with future asthma exacerbation. In that way, EBC urates could be proposed as marker of current impairment (frequency of symptoms), as well as markers of risk (likelihood of asthma exacerbation).

Asthma is chronic inflammatory disease of the airways in which systemic inflammation also play an important role [33]. However, the role of systemic biomarkers from peripheral blood in asthmatics is still poorly investigated. Serum hs-CRP is a biomarker of low grade systemic inflammation. It is increased in uncontrolled asthmatics and correlate with local inflammation (FEV1, EBT) and asthma symptoms [33]. It is assumed that systemic biomarkers are insufficient in representing local inflammatory process in asthma. Lewis and others have shown that monocytes are predominantly associated with symptoms indicative of obstructive airway disease, in similar relation to neutrophils, but both of these leukocyte counts are also increased in asthma patients in older age groups [34]. In our study, A-monocyte Z-score significantly correlated with frequency of asthma symptoms confirming the findings of Lewis et al. These results highlight monocytes as a marker of chronic systemic inflammation in asthma.

We are aware of certain limitations of this study: the lack of data on other biomarkers of acid and oxidative stress, the lack of EBC dilution markers and data of dilutions of EBC itself, also asthma exacerbation was self-reported by the patient.

In conclusion, the study of combination of various exhaled breath and exhaled breath condensate BMs should continue, especially in longitudinal studies with repeated measurements of biomarkers. Predictive biomarkers may be the basis for individualized therapy of asthmatic children.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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