Eicosanoids in exhaled air after non-specific challenge in patients with asthma

Marek Kaszuba¹, Jagoda Kaszuba¹, Agata Sawina¹, Agata Kleciek¹, Paweł Stręk², Marek Sanak¹ and Lucyna Mastalerz¹
*Correspondence: markas@op.pl
¹Second Department of Internal Medicine, Jagiellonian University Medical College, Poland.
²Department of Otolaryngology, Jagiellonian University Medical College, Poland.

Abstract

Background: Histamine is used as a direct stimulus to measure airway responsiveness. This short-acting biogenic amine acts mainly on airway smooth muscle receptors causing bronchoconstriction and is used for the airway hyperresponsiveness assessment in asthmatic patients. In aspirin-induced asthma (AIA) special regulatory role of eicosanoids is postulated. The aim of the study was to assess the influence of histamine on a wide profile of eicosanoids measured in exhaled breath condensate (EBC) in AIA patients and asthmatics tolerating aspirin well (ATA).

Methods: The study population consisted of seventeen asthmatics. Ten of them were AIA patients. Eicosanoid concentrations in EBC were determined using gas chromatography/mass spectrometry or high-performance liquid chromatography/tandem mass spectrometry. Measurements were performed at baseline and following bronchial histamine challenge.

Results: Bronchial reactions were precipitated by histamine in all patients and accompanied only by decrease of leukotriene (LT) C₄ and trans-LTC₄ mean level. The AIA group was characterized by higher levels of cysteinyl leukotrienes, LTC₄, and prostaglandin (PG) E₂ in EBC at baseline, and decrease in EBC concentration of LTC₄, trans-LTC₄ and tetranor-PGE-M following histamine challenge. In the ATA group no significant changes in eicosanoids levels after histamine were noticed.

Conclusions: AIA patients present different baseline profile of EBC eicosanoids in comparison to patients with ATA. Histamine administered locally during a bronchial challenge test may influence inflammatory mediators and thus trigger indirect effects in the respiratory tract. This response for histamine differentiates two studied phenotypes of asthma; only in the AIA group histamine precipitates alterations of the eicosanoid synthesis in the lungs.

Keywords: Aspirin, asthma, hypersensitivity, eicosanoids, histamine

Introduction

Histamine is a short-acting biogenic amine which plays an important role in allergic inflammation. Mast cells and basophiles store large quantities of histamine, which is released during degranulation in response to immunologic and non-immunologic stimuli [1]. Histamine elicits many biological effects acting through four types of histamine receptors located on cells of various types. These include airway smooth muscle cells provoking airflow obstruction and sensory fibers causing a reflex response that can induce bronchospasm. Histamine, beside methacholine, is used as a direct stimulus to measure airway responsiveness.

Airway hyperresponsiveness (AHR) is one of the clinical features of asthma. Its measurement is crucial for establishing the correct diagnosis [17]. The components of airway changes in asthma that contribute to AHR can be differentiated into two categories: airway remodeling and airway inflammation.

It has been suggested that the action of histamine particularly reflects the effect associated with contractility of airway smooth muscle, called a direct effect. To a lesser degree, histamine may affect airway inflammation, which is called an indirect effect. However, the role of histamine in direct and indirect effects has not been clearly established. There is a correlation between measurements of airway responsiveness and number of eosinophils, mast cells [13] and epithelial cells [4] in bronchoalveolar lavage fluid (BALF) in asthma. In our study, we focused on the indirect action of histamine by measuring mediators released from inflammatory cells into the lumen of the airways in response to this agent.

Eicosanoids, including prostaglandins and leukotrienes, are arachidonic acid metabolites which play an important role in chronic inflammation in asthma, especially in the pathogenesis of aspirin intolerant asthma (AIA). AIA is a distinct clinical syndrome which affects 5-10% of asthmatics and refers to the coexistence of hypersensitivity to non-steroidal anti-inflammatory drugs, rhinosinusitis/nasal polyps, and asthma [30].

Prostaglandin (PG) E₂ is the most abundant prostanooid in the lower respiratory tract, produced mainly by epithelial and airway smooth muscle cells. Multiple studies have shown that PGE₂ attenuates bronchoconstriction, increases the relaxation of airway smooth muscles and inhibits mast-cell degranulation [26]. PGE₂ activity is expressed not only by direct effect on bronchial smooth muscle relaxation but also as suppression of the inflammatory mediators releasing mainly by eosinophils and mast cells [32]. In AIA diminished level of PGE₂ was found in nasal polyps [15], peripheral blood cells [27] and bronchial...
fibroblasts [19]. On the contrary, PGD$_2$, mast cell autacoid mediator, is a potent agonist for airway smooth muscle contraction. The PGE$_2$ and PGD$_2$ mediators are chemically unstable and become rapidly converted, via 13,14-dihydro-15-keto-PGE$_2$ and 9α,11β-PGF$_2$, respectively, into tetranor-PGE-M and tetranor-PGD-M. The latter stable metabolites are considered to be the markers of PGE, and PGD$_2$ systemic production. One of the ultimate dehydration product of PGD$_2$ is 15-deoxy-delta$_{12,14}$-PGJ$_2$, whose level increases under stress such as infection and inflammation.

The inflammatory process in asthma is strongly influenced by leukotriene driven pathways. Leukotriene (LT) C$_4$, LTD$_4$, LTE$_4$ are often called cysteinyl leukotrienes (CysLTs). Trans-LTC$_4$ is an isomer of LTC$_4$ [12]. The increased CysLTs level, which may be the result of chronic eosinophilic inflammation of upper and lower respiratory tract, is considered a hallmark of AIA. LTE$_4$, the end-metabolite of CysLTs, is a reliable marker of leukotriene generation in the lung.

Eicosanoids are present in very low concentrations in body fluids and exhaled air. Exhaled breath condensate (EBC) is a non-invasive technique for monitoring airway inflammation in inflammatory diseases such as asthma. Mediators measured in EBC are believed to derive from epithelial lining fluid of the small airways and alveoli [10].

The aim of our study was to evaluate the changes in concentration of wide eicosanoid spectrum in EBC during asthmatic response following histamine inhalation. We used this non-specific agent to assess its influence on inflammation in the airways. The study was carried out in patients with AIA and aspirin-tolerant asthma (ATA). We wondered if clinically manifested bronchoconstriction was accompanied by any change in eicosanoids profile in EBC in asthmatics. To our knowledge, we are the first to perform such investigations.

**Material and methods**

**Study subjects**
The study population consisted of 17 asthmatics: 10 AIA subjects and 7 ATA patients. The diagnosis of asthma was established according to Global Initiative for Asthma 2011 update [3]. Hypersensitivity to aspirin was confirmed by an oral aspirin provocation test within 12 months before the study. All ATA patients occasionally used aspirin without any adverse reactions.

Study participants had stable asthma without any exacerbation or respiratory tract infection during 6 weeks preceding the study. Their baseline FEV$_1$ (forced expiratory volume in 1 second) was >70% of predicted value on the day of the study. The subjects were instructed to withdraw medications that decrease bronchial responsiveness before the histamine challenge. Inhaled corticosteroids were allowed at the dose ≤1500 μg of fluticasone equivalent per day. None of the patients was treated with systemic corticosteroids. The characteristics of the patients are presented in (Table 1).

All the subjects gave informed consent. The study was approved by the University Ethics Committee (KBET/44/B/2009).

**Study design**
The single-blind bronchial challenge with histamine [20] began with inhalation of 5 breaths of placebo (saline). Histamine was inhaled every 10 minutes in increasing doses to the cumulative dose 2530 μg. FEV$_1$ was measured before, 3 minutes after placebo inhalation and every 3 minutes after each dose of histamine. The challenge procedure was interrupted if FEV$_1$ decreased by more than 20% of the postsaline baseline value, or if the cumulative dose of histamine was reached. The cumulative dose of histamine causing bronchoconstriction (expressed as 20% fall in FEV$_1$) was recorded as provocative dose (PD20). FEV$_1$ was assessed after the last dose of histamine every 30 min., until FEV$_1$ reached >80% postsaline baseline value.

In all study participants the histamine challenge was positive and EBC was collected within 30 min. before the challenge and during 30 min. after the time of appearance of bronchoconstriction which was restored spontaneously or was reversed by the inhalation of short-acting $\beta_2$-agonist.

**Lung function**
Pulmonary function tests were performed using a flow-integrating computerized pneumotachograph (Pneumoscreen, E. Jaeger, Germany).

**Exhaled breath condensate**
EBC was collected according to the American Thoracic Society/European Respiratory Society guidelines [10] using EcoScreen instrument (GmbH Hoechberg, Germany). During 20 minutes of tidal breathing, 2-3 ml of clear fluid was collected and immediately deep frozen.

**Biochemical assays**
Concentration of eicosanoids in exhaled breath condensate was measured using gas chromatography/mass spectrometry (GC-MS) and high-performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) according to the methodology described elsewhere [24,25]. The results were reported in picograms per milliliter of EBC (pg/ml), or recalculated as picograms per microgram of palmitic acid content (pg/μg of PA) due to variable dilution by condensed water.

**Statistical analysis**
Descriptive statistics were expressed as mean, standard deviation, median, lower and higher quartile (25% and 75% percentile). General linear model including repeated measures analysis of variance was used for multiple comparisons. Logarithmic transformation was used when needed as variance stabilizing transformation. Correlation between variables was estimated using the Spearman rank order correlations.
Results
Clinical reactions
During histamine challenge none of the study subjects developed any symptoms after placebo inhalation. All participants responded to histamine. The only statistically significant differences in clinical characteristics between the subjects with AIA and with ATA were higher blood eosinophil count (p=0.03), and lower PD20 (p=0.008) in the AIA group (Table 1).

Exhaled breath condensate concentration of eicosanoids
In the studied asthematics, LTC₄ and its isomer trans-LTC₄ proved to be the only measured eicosanoids for which EBC concentrations changed following the histamine challenge (Table 2). After the administration of inhaled histamine, their EBC concentration decreased significantly.

At baseline, the AIA group was characterized by higher CysLTs, LTC₄, and PGE₂ levels in EBC (Figure 1). Moreover, 15-deoxy-delta12,14-PGJ₂ row baseline concentration in EBC was increased in the AIA group (Table 3). Also, the assessment of changes in eicosanoids EBC concentration after administering histamine enabled us to observe differences between the AIA and ATA groups (Table 3). Following the histamine challenge, the levels of LTC₄ (Figure 2), trans-LTC₄ (Figure 3) and tetranor-PGE-M (Figure 4) decreased in the AIA group. None of the measured eicosanoids changed its level after histamine in ATA group.

Correlation between clinical variables and EBC eicosanoids
Correlation analysis in subgroups was not performed due to the small number of subjects in the groups.

In all studied asthmatics a significant positive correlation between peripheral blood eosinophils and baseline PGE₂ (r=0.69) and LTC₄ (r=0.61) concentration in EBC was observed. Baseline FEV1 was negatively correlated with post-challenge LTE₄ (r=-0.63) and CysLTs (r=-0.67) EBC concentration. Pre-challenge PGE₂ correlated significantly with post-challenge one (r=0.60). No significant correlation was found between baseline and post-challenge EBC concentration within other

\[ \text{PD20 (mg of histamine)} \]

\[ 926.5±709.2 \]

\[ 414.29±320.38 \]

\[ 1285±692.07 \]

\[ 1100(960-1850) \]

\[ * \text{ statistical significance values are expressed as mean±SD and median with upper and lower quartile (25% and 75% percentiles)} \]

\[ \text{ACT: Asthma Control Test; AIA: aspirin-induced asthma, ATA: aspirin-tolerant asthma, FEV1: forced expiratory volume in 1 second, IgE: immunoglobulin E, IU: international unit, N.S: not significant, NHLBI: The National Heart, Lung, and Blood Institute; no., number; PD20: provocative dose; y, year.} \]

\[ \text{Table 1. Clinical characteristics of the patients in the subgroups.}\]

|               | AIA+ATA (n=17) | AIA (n=7) | ATA (n=10) | p-value AIA vs. ATA |
|---------------|----------------|-----------|------------|-------------------|
| Age (yr.)     | 45.9±11.7      | 45.3±11.9 | 46.3±12.2  | 0.87              |
|               | 45 (35-56)     | 46 (34-57)| 44(39-56)  |                   |
| Female / Male. no. | 10/7         | 2/5       | 8/2        | 0.051             |
| Duration of asthma (yr.) | 7.82±6.89   | 8.73±5.15 | 7.2±8.11   | 0.28              |
|               | 6 (3-10)       | 6(5-14)   | 3.5(1-10)  |                   |
| Inhaled steroids (yes/no), no. | 11/6        | 1/6       | 5/5        | 0.31              |
| Inhaled steroids ( g of fluticasone equivalent/d) | 426.5±490.3 | 678.6±590.1 | 250±333.33 | 0.08              |
| FEV1 baseline (% predicted) | 89.64±10.58 | 86.79±10.06 | 91.64±11 | 0.37              |
|               | 89.56(82.77-94.44) | 85.26(80.53-93.24) | 94.25(92.77-97.2) |                   |
| Total IgE (IU/mL) | 100.9±149.4 | 69.4±30.35 | 122.8±194.35 | 0.46              |
| Blood eosinophil count (mm-3) | 315.9±224.9 | 477.57±261.08 | 202.8±99.58 | 0.03*             |
|               | 284(200-339) | 484(284-792) | 223.5(121-285) |                   |
| NHLBI asthma grade | 1.29±0.47 | 1.4±0.53 | 1.2±0.42 | 0.35              |
|               | 1(1-2)         | 1(1-2)    | 1(1-1)     |                   |
| ACT | 22.71±3.35 | 20.7±4.46 | 24.1±1.2 | 0.13              |
|               | 24(22-25)      | 22(16-25) | 24.5(24-25) |                   |
| PD20 (mg of histamine) | 926.5±709.2 | 414.29±320.38 | 1285±692.07 | 0.008*            |
|               | 960(350-1100) | 350(155-505) | 1100(960-1850) |                   |

* statistical significance values are expressed as mean±SD and median with upper and lower quartile (25% and 75% percentiles) ACT: Asthma Control Test; AIA: aspirin-induced asthma, ATA: aspirin-tolerant asthma, FEV1: forced expiratory volume in 1 second, IgE: immunoglobulin E, IU: international unit, N.S: not significant, NHLBI: The National Heart, Lung, and Blood Institute; no., number; PD20: provocative dose; y, year.
Table 2. Eicosanoids in asthmatic patients at baseline and following histamine challenge.

| Eicosanoid   | baseline | challenge | p-value |
|--------------|----------|-----------|---------|
| LTB₄         | 48.40    | 35.57     | 0.22    |
| [pg/mL]      | (33.28-55.28) | (24.37-52.09) |  |
| LTB₃         | 74.64    | 70.42     | 0.71    |
| [pg/μg of PA] | (62.88-94.13) | (42.01-119.02) |  |
| CysLTs       | 5.44     | 4.53      | 0.21    |
| [pg/mL]      | (4.52-7.68) | (3.79-6.42) |  |
| CysLTs       | 9.55     | 9.33      | 0.33    |
| [pg/μg of PA] | (8.32-13.72) | (6.99-11.45) |  |
| LTC₄         | 1.71     | 1.14      | 0.04*   |
| [pg/mL]      | (1.21-2.14) | (0.76-1.53) |  |
| LTC₄         | 2.62     | 2.05      | 0.10    |
| [pg/μg of PA] | (2.13-3.44) | (1.19-3.42) |  |
| trans-LTC₄   | 1.24     | 0.62      | 0.02*   |
| [pg/mL]      | (0.74-2.49) | (0.53-1.11) |  |
| trans-LTC₄   | 2.23     | 1.62      | 0.03*   |
| [pg/μg of PA] | (1.47-3.71) | (0.75-2.35) |  |
| LTD₄         | 1.09     | 0.93      | 0.64    |
| [pg/mL]      | (0.77-1.23) | (0.72-1.23) |  |
| LTD₄         | 1.80     | 1.45      | 0.71    |
| [pg/μg of PA] | (1.12-2.25) | (1.21-2.45) |  |
| PGE₂         | 2.86     | 2.23      | 0.31    |
| [pg/mL]      | (1.93-4.18) | (1.62-3.40) |  |
| PGE₂         | 5.22     | 4.33      | 0.76    |
| [pg/μg of PA] | (3.49-6.74) | (3.09-6.18) |  |
| PGE₂         | 3.29     | 4.11      | 0.29    |
| [pg/μg of PA] | (2.73-4.65) | (3.32-5.18) |  |
| 13,14-dihydro-15-keto-PGE₂ | 3.49 | 3.83 | 0.67 |
| [pg/mL] | (2.72-4.88) | (2.76-4.86) |  |
| 13,14-dihydro-15-keto-PGE₂ | 5.03 | 6.34 | 0.59 |
| [pg/μg of PA] | (4.44-8.03) | (4.77-9.60) |  |
| tetranor-PGE-M | 21.36 | 15.76 | 0.41 |
| [pg/mL] | (12.20-38.80) | (8.80-23.77) |  |
| tetranor-PGE-M | 34.50 | 27.64 | 0.71 |
| [pg/μg of PA] | (25.82-49.50) | (16.26-45.38) |  |
| PGD₂         | 1.57     | 1.40      | 0.67    |
| [pg/mL]      | (1.29-1.77) | (1.00-1.85) |  |
| PGD₂         | 2.12     | 2.09      | 0.24    |
| [pg/μg of PA] | (1.86-3.03) | (1.94-3.49) |  |

Continuation of Table 2.

| Eicosanoid   | baseline | challenge | p-value |
|--------------|----------|-----------|---------|
| 13,14-dihydro-15-keto-PGD₂ | 3.89 | 3.79 | 0.85 |
| [pg/mL]      | (2.44-5.03) | (2.44-4.90) |  |
| 13,14-dihydro-15-keto-PGD₂ | 6.27 | 5.88 | 0.40 |
| [pg/μg of PA] | (4.23-7.44) | (4.56-8.71) |  |
| 9α,11b-PGF₂ | 0.17 | 0.11 | 0.14 |
| [pg/mL] (GC-MS) | (0.13-0.34) | (0.09-0.26) |  |
| 9α,11b-PGF₂ | 0.28 | 0.22 | 0.42 |
| [pg/μg of PA] | (0.22-0.65) | (0.16-0.53) |  |
| 15-deoxy-delta12,14-PGJ₂ | 3.58 | 2.88 | 0.82 |
| [pg/mL] | (1.65-5.06) | (2.34-4.98) |  |
| 15-deoxy-delta12,14-PGJ₂ | 5.54 | 6.87 | 0.60 |
| [pg/μg of PA] | (3.90-8.60) | (5.10-8.13) |  |

* statistical significance values are expressed as median with upper and lower quartile [25%-75% percentiles]

AIA: aspirin-induced asthma, ATA: aspirin-tolerant asthma, CysLTs: cysteinyl leukotrienes, GC-MS: gas chromatography/mass spectrometry, LT: leukotriene, PA: palmitic acid, PG: prostaglandin.

Discussion

At the beginning of the discussion section, some limitations of the study need to be noted. The main limitation consisted in relatively small patient populations. Another one was the lack of healthy control group. Such a group in the study could have helped us to better assess the production of eicosanoids in the respiratory system of the asthmatics. The study focused mainly on distinguishing between AIA and ATA in this respect.

We have noticed positive significant correlation between peripheral eosinophilia and baseline bronchial production of LTC₄ in the studied patients.

The higher level of blood eosinophils was observed in the AIA group, which can probably be connected with decreased eosinophil apoptosis [33]. This observation is in line with previous reports [11,31]. The studied AIA subgroup was also characterized by increased baseline concentration of CysLTs and LTC₄ in EBC. It is consistent with earlier study using immunoassay [2], although other studies using spectrometric methods did not demonstrate baseline overproduction of CysLTs [22] or LTC₄ [24] in the airways in AIA. However, our observation is in line with researches assessing LTC₄ level in induced sputum [18] and can be related to overexpression of LTC synthase (LTCS) in eosinophils [23] and bronchi [21] of asthmatics with aspirin hypersensitivity. Thus, overproduction...
### Table 3. Eicosanoids in asthmatic patients at baseline and following histamine challenge in the subgroups.

|            | AIA              | ATA              | p-value |
|------------|------------------|------------------|---------|
|            | baseline | challenge | baseline | challenge | I   | II   | III  |
| **LTB₄**  | 50.37    | 44.16    | 39.53    | 30.66    | 0.39 | 0.74 | 0.90 |
| [pg/mL]    | (38.39-97.38) | (24.37-59.99) | (25.11-55.28) | (11.44-47.89) |     |      |      |
| **LTB₃**  | 83.00    | 78.53    | 71.11    | 60.56    | 0.56 | 0.93 | 1.00 |
| [pg/μg of PA] | (62.88-173.98) | (42.01-119.02) | (54.81-94.13) | (30.56-120.39) |     |      |      |
| **CysLTs**| 7.68     | 4.57     | 4.67     | 4.48     | 0.01*| 0.07 | 0.99 |
| [pg/mL]    | (5.62-14.46) | (4.2-6.42)  | (3.52-5.44) | (3.04-6.5)   |     |      |      |
| **CysLTs**| 13.72    | 9.85     | 8.46     | 8.6      | 0.02*| 0.13 | 0.63 |
| [pg/μg of PA] | (9.55-19.94) | (4.97-11.45)| (6.19-10.66) | (7.23-11.63)|     |      |      |
| **LTC₄**  | 2.24     | 0.87     | 1.22     | 1.39     | 0.003*| 0.001*| 1.00 |
| [pg/mL]    | (1.90-5.19) | (0.54-1.43) | (0.91-1.44) | (0.76-1.77)  |     |      |      |
| **LTC₄**  | 3.68     | 1.83     | 2.15     | 2.60     | 0.01*| 0.002*| 0.88 |
| [pg/μg of PA] | (3.32-6.62) | (0.83-2.26) | (1.69-2.61) | (1.89-3.71)  |     |      |      |
| **trans-LTC₄** | 2.93 | 0.54 | 0.88 | 1.10 | 0.19 | 0.001* | 0.83 |
| [pg/mL]    | (0.60-4.72) | (0.48-0.62) | (0.74-1.37) | (0.60-1.52)  |     |      |      |
| **trans-LTC₄** | 4.81 | 0.85 | 2.01 | 2.27 | 0.19 | 0.001* | 0.97 |
| [pg/μg of PA] | (1.07-5.88) | (0.62-1.36) | (1.47-2.60) | (1.62-2.94)  |     |      |      |
| **LTD₄**  | 1.19     | 1.12     | 0.96     | 0.80     | 0.59 | 1.00 | 1.00 |
| [pg/mL]    | (0.88-1.65) | (0.83-1.76) | (0.75-1.15) | (0.72-1.07)  |     |      |      |
| **LTD₄**  | 1.80     | 1.84     | 1.96     | 1.42     | 0.91 | 0.99 | 0.99 |
| [pg/μg of PA] | (1.12-2.94) | (1.28-2.72) | (0.92-2.25) | (1.20-2.16)  |     |      |      |
| **LTE₄**  | 4.18     | 2.80     | 2.31     | 1.79     | 0.22 | 0.58 | 0.97 |
| [pg/mL]    | (3.18-7.62) | (2.21-3.40) | (1.49-2.86) | (1.48-4.11)  |     |      |      |
| **PGE₂**  | 6.74     | 6.08     | 4.08     | 4.32     | 0.42 | 0.73 | 1.00 |
| [pg/μg of PA] | (5.22-9.50) | (2.58-6.74) | (3.17-5.79) | (3.09-5.93)  |     |      |      |
| **PGE₂**  | 3.22     | 2.84     | 1.58     | 1.58     | 0.004*| 0.96 | 1.00 |
| [pg/mL]    | (2.23-4.13) | (2.21-7.89) | (1.24-1.94) | (1.15-2.00)  |     |      |      |
| **PGE₂**  | 4.90     | 5.04     | 2.76     | 3.68     | 0.04*| 0.80 | 0.90 |
| [pg/μg of PA] | (3.66-7.08) | (4.11-11.56)| (2.29-3.29) | (2.33-4.34)  |     |      |      |
| **13,14-dihydro-15-keto-PGE₂** | 3.82 | 3.83 | 2.73 | 3.46 | 0.83 | 0.91 | 1.00 |
| [pg/mL]    | (3.49-4.88) | (2.96-4.35) | (2.26-5.65) | (2.50-5.41)  |     |      |      |
| **13,14-dihydro-15-keto-PGE₂** | 6.55 | 6.34 | 4.64 | 6.22 | 1.00 | 1.00 | 0.88 |
| [pg/μg of PA] | (4.65-8.03) | (3.81-8.73) | (3.32-15.98) | (4.77-15.88)  |     |      |      |
| **tetranor-PGE-M** | 22.88 | 8.80 | 12.36 | 20.79 | 0.41 | 0.02*| 0.23 |
| [pg/mL]    | (16.87-38.80) | (8.39-11.14) | (7.97-38.89) | (15.76-32.78) |     |      |      |
| **tetranor-PGE-M** | 36.64 | 14.56 | 33.41 | 41.25 | 0.73 | 0.04*| 0.10 |
| [pg/μg of PA] | (28.50-49.50) | (13.00-19.42)| (10.92-51.85)| (27.64-87.07)|     |      |      |
| **PGD₂**  | 1.48     | 1.48     | 1.58     | 1.23     | 0.99 | 0.99 | 0.90 |
| [pg/mL]    | (1.29-1.77) | (1.20-2.36) | (1.28-1.86) | (0.84-1.85)  |     |      |      |
| **PGD₂**  | 2.52     | 2.29     | 2.04     | 1.98     | 0.099 | 0.86 | 1.00 |
| [pg/μg of PA] | (1.89-3.03) | (1.99-5.19) | (1.76-4.80) | (1.64-3.49)  |     |      |      |
| **13,14-dihydro-15-keto-PGD₂** | 5.03 | 4.81 | 2.91 | 2.92 | 0.24 | 0.93 | 0.99 |
| [pg/mL]    | (4.19-5.63) | (3.52-4.92) | (1.95-3.48) | (2.16-4.21)  |     |      |      |
| **13,14-dihydro-15-keto-PGD₂** | 7.18 | 6.64 | 4.97 | 5.88 | 0.82 | 1.00 | 0.67 |
Continuation of Table 3.

|                  | AIA         | ATA         | p-value |
|------------------|-------------|-------------|---------|
|                  | baseline    | challenge   | baseline | challenge | I  | II  | III |
| [pg/ug of PA]    | (6.27-9.33) | (5.44-9.86) | (4.04-7.44) | (3.90-8.71) |
| 9a,11b-PGF \_2   | 0.22        | 0.10        | 0.16     | 0.12      | 0.88 | 0.91 | 0.52 |
| [pg/mL] (GC-MS)  | (0.14-0.38) | (0.08-0.71) | (0.09-0.28) | (0.09-0.14) |
| 9a,11b-PGF \_2   | 0.30        | 0.20        | 0.24     | 0.23      | 0.99 | 0.99 | 0.85 |
| [pg/ug of PA]    | (0.25-0.65) | (0.14-1.23) | (0.18-0.70) | (0.16-0.39) |
| 15-deoxy-delta12,14-PGJ \_2 | 5.55 | 3.30 | 2.55 | 2.84 | 0.04* | 0.43 | 0.41 |
| [pg/mL] (GC-MS)  | (4.24-8.36) | (2.12-4.98) | (1.43-3.58) | (2.34-5.02) |
| 15-deoxy-delta12,14-PGJ \_2 | 9.13 | 5.40 | 4.58 | 7.11 | 0.09 | 0.73 | 0.32 |
| [pg/ug of PA]    | (5.28-10.66)| (3.66-8.33) | (2.52-5.84) | (5.51-8.13) |

* statistical significance values are expressed as median with upper and lower quartile [25%-75% percentiles]

AIA: aspirin-induced asthma, ATA: aspirin-tolerant asthma, CysLTs: cysteinyl leukotrienes, GC-MS: gas chromatography/mass spectrometry, LT: leukotriene, PA: palmitic acid, PG: prostaglandin.

p-values:
I - AIA vs ATA at baseline.
II – AIA at baseline vs AIA post challenge.
III – ATA at baseline vs ATA post challenge.

Figure 1. (a, b)Eicosanoids concentrations in EBC. AIA: aspirin induced asthma, ATA: aspirin tolerant asthma, EBC: exhaled breath condensate, post: after histamine, pre: before histamine; *, p<0.05.

Figure 2. Changes in LTC4 concentration in EBC during bronchial challenge with histamine. AIA: aspirin induced asthma, ATA: aspirin tolerant asthma, EBC: exhaled breath condensate, LT: leukotriene, post: after histamine, pre: before histamine; *, p<0.05.

Figure 3. Changes in trans-LTC4 concentration in EBC during bronchial challenge with histamine. AIA: aspirin induced asthma, ATA: aspirin tolerant asthma, EBC: exhaled breath condensate, trans-LT: trans-leukotriene, post: after histamine, pre: before histamine; *, p<0.05.
of LTC4 observed in our study in AIA may be the result of both overexpression of LTC4 and overrepresentation of eosinophils. Other important sources of eicosanoids could also be taken into consideration while explaining these observations, such as mast cells and airway epithelial cells, which were not assessed in the study.

The elevated level of PGE2 in EBC in the AIA group was a surprising finding. Other publications suggest diminished production of PGE2 in peripheral blood cells, upper airways and bronchial fibroblasts [30] in AIA. However the level of PGE2 in BALF after placebo inhalation was higher in patients with AIA [28]. Increased production of PGE2 by basophil-enriched leukocytes in AIA patients has also been reported [8].

Bronchoconstriction appeared in both groups, which probably is an effect of direct histamine action on airway smooth muscle via histamine receptors. Interestingly, the level of PD20 was much lower in AIA than in ATA group. It may indicate more severe bronchial inflammation in asthmatics sensitive to aspirin. The elevated levels of CysLTs, LTC4, PGE2 and 15-deoxy-delta12,14-PGJ2 in EBC of the AIA patients can be observed in our study as the result of such a condition. This state can be mirrored also in reported more severe bronchial inflammation in asthmatics after inhalation of histamine. This indirect action of histamine let us to reveal discrepancies in asthmatic inflammation between subjects with hypersensitivity to aspirin and tolerating aspirin well.

Conclusions
The aim of this study was to assess the indirect effect of locally administrated stimulus on lower airways by measuring wide profile of eicosanoids released to the bronchi and present in EBC during the bronchial histamine challenge. In our study we demonstrated that histamine triggers not only direct but also indirect effects in respiratory tract causing both bronchoconstriction and alterations in the eicosanoid synthesis in the lungs. We demonstrated for the first time changes in exhaled eicosanoids in asthmatics after inhalation of histamine. This indirect action of histamine let us to reveal discrepancies in asthmatic inflammation between subjects with hypersensitivity to aspirin and tolerating aspirin well.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions

| Authors’ contributions | MK | JK | AS | AK | PS | MS | LM |
|------------------------|----|----|----|----|----|----|----|
| Research concept and design | ✓ | | | | ✓ | | ✓ |
| Collection and/or assembly of data | ✓ | | ✓ | ✓ | ✓ | | |
| Data analysis and interpretation | | ✓ | | | | | |
| Writing the article | ✓ | ✓ | | | | | |
| Critical revision of the article | | | | ✓ | ✓ | | |
| Final approval of article | | | | | | | |
| Statistical analysis | ✓ | | | | | | |

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