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Implications of prostaglandin D2 and leukotrienes in exhaled breath condensates of asthma

Yoshitaka Uchida, MD^{*,†}; Tomoyuki Soma, MD, PhD^{*,†};
Kazuyuki Nakagome, MD, PhD^{*,†}; Takehito Kobayashi, MD, PhD^{*,†};
Makoto Nagata, MD, PhD^{*,†}

^{*} Department of Respiratory Medicine, Saitama Medical University, Iruma-gun, Saitama, Japan

[†] Allergy Center, Saitama Medical University Hospital, Iruma-gun, Saitama, Japan

[‡] Department of General Internal Medicine, Saitama Medical University, Iruma-gun, Saitama, Japan

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ABSTRACT

Background: Various inflammatory eicosanoid levels in biomaterials from airways of asthma and their associations with clinical parameters remain uncertain. We hypothesized that prostaglandin and leukotriene levels differ between in exhaled breath condensates (EBCs) and in sputum in mild, moderate, and severe levels of asthma and that EBC and sputum eicosanoid levels are associated with indexes of pulmonary function and inflammation.

Objective: To determine the differences between EBC and sputum eicosanoid levels in healthy participants and patients with asthma with different asthma severity levels.

Methods: Collected EBC and sputum, as well as pulmonary function, were examined in adult patients with asthma and healthy participants. Exhaled breath condensate prostaglandin D2-methoxime (PGD2-MOX), cysteinyl leukotrienes (CysLTs), leukotriene B4 (LTB4), and thromboxane B2 levels, and some sputum eicosanoid and tryptase levels were measured. Differences in eicosanoid levels among participants and their associations with pulmonary function and tryptase and granulocyte levels in sputum were then evaluated.

Results: Analysis of 94 EBCs and 43 sputa revealed that EBC and sputum PGD2-MOX and CysLT levels were significantly higher in patients with asthma than in healthy participants. Exhaled breath condensate PGD2-MOX, CysLT, and LTB4 levels were significantly higher in patients with severe asthma. Exhaled breath condensate PGD2-MOX level was also significantly correlated with sputum tryptase levels and lower pulmonary function in patients with asthma. Sputum PGD2-MOX and CysLT levels were significantly correlated with the proportion of eosinophils among all cells in sputum in patients with asthma.

Conclusion: The results suggest that EBC PGD2 levels are associated with impairment of pulmonary function in adults with asthma who have undergone guideline treatment. Exhaled breath condensate or sputum PGD2 and CysLTs may represent severity or airway inflammation in asthma.

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Introduction

Inflammatory eicosanoids, including prostanoids, leukotrienes (LTs), and thromboxanes,^{1–3} are crucial mediators in asthma. Prostaglandin D2 (PGD2), a major inflammatory prostanoid produced by activated mast cells, and LTC4, LTD4, and LTE4, which are

known as cysteinyl leukotrienes (CysLTs), cause contraction of airway smooth muscle and promote granulocyte and lymphocyte infiltration and activation, leading to type 2 airway inflammation and remodeling in asthma.^{1–3} Leukotriene B4 (LTB4) causes a weak bronchial contraction and promotes neutrophil activation, which contributes to airway edema and mucus secretion.¹ Thromboxane A2, a prostanoid, is involved in allergic airway inflammation in vitro.⁴ Recently, evidence has emerged of the involvement of PGD2 and CysLTs in the pathophysiology of severe asthma.^{5–18} Prostaglandin D2 is increased in the bronchoalveolar lavage fluid (BALF) and associated with higher numbers of mast cells in the bronchial wall in cases with severe asthma.^{5,6} Increases in mast

Reprints: Tomoyuki Soma, MD, PhD, Department of Respiratory Medicine and Allergy Center, Saitama Medical University, Iruma-gun, Saitama 350-0495, Japan; E-mail: tsoma@saitama-med.ac.jp.

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cells in the bronchial wall/the alveolar parenchyma may result in uncontrolled asthma or asthma exacerbation.^{6–8} Prostaglandin D2 and CysLTs also are involved in innate lymphoid cell type 2 (ILC2s) activation,^{9–12} which shows high expression of the chemo-attractant receptor–homologous molecule expressed on TH2 lymphocytes (CRTH2/DP2), a receptor of the PGD2, in severe asthma.^{10,13} The CRTH2/DP2 levels are increased in poorly controlled asthma.⁶ Cysteinyl leukotrienes are also increased in exhaled breath condensates (EBCs) in severe asthma^{14,15} and possibly involved in airway remodeling in asthma.^{16–18}

Despite this evidence, clinical contributions of these lipid mediators to chronic asthma have hardly been elucidated. Several studies have shown that prostanoids in the EBC, sputum, and BAL in patients with asthma are at levels similar to those in healthy participants, and their levels in patients with asthma are not associated with asthma severity levels.^{19–22} Conversely, a few studies using bronchoscopy, an invasive procedure, demonstrated different levels of PGD2 in BALF among patients with mild to severe asthma.^{5,6} Exhaled breath condensates, sputum, or BALF LT levels are higher in patients with asthma than in healthy participants, but various findings on their differences depending on asthma severity have been reported.^{14,15,23–26} No chronic detrimental effects of PGD2^{19,20,27} and CysLTs^{14,24,26} on lung function were observed previously. Thus, discrepancies are seen between the positive results of experimental studies that showed effects of PGD2 and CysLTs on ILC2 activation and involvement of CysLTs in airway remodeling, and the diverse results of clinical studies that found various PGD2 and CysLT levels in patients with asthma and no association of these eicosanoids with physiological markers of asthma. This led us to further examine eicosanoids in EBC and sputum.

Therefore, we hypothesized that patients with asthma had increased levels of inflammatory prostanoids and LT in the airways, that the levels varied with different asthma severity levels, and that these eicosanoids are associated with clinical and pathophysiological parameters of asthma. The study aimed to determine EBC and sputum PGD2, CysLTs, LTB4, and thromboxane B2 (TXB2) levels in patients with asthma with different asthma severity levels and evaluate their associations with pulmonary function, tryptase, and inflammatory cells in sputum. We measured eicosanoid levels in sputum to confirm previous results obtained in EBC and to determine the association between eicosanoids and airway inflammatory cells.

Methods

Patients and Study Design

Outpatients with asthma and healthy control participants aged 20 to 80 years were recruited from the Allergy Centre of Saitama Medical University Hospital. Asthma was diagnosed based on the presence of a clinical history regarding asthma (symptom, history of exacerbation, and physical examination) and bronchial reversibility reflected by bronchodilator responsiveness or bronchial hyperresponsiveness using methacholine challenge, which satisfied the Global Initiative for Asthma (GINA) guidelines.²⁸ Outpatients with asthma were classified as having mild (steps 1 and 2), moderate (steps 3 and 4), and severe asthma (step 5), according to the GINA guidelines. Participants satisfying the following criteria were excluded: other pulmonary diseases; asthma exacerbation or respiratory infections during the 4 weeks before the initial day of this study; pregnancy; severe comorbid diseases, such as cancer, renal failure/heart failure; and current smoker or a smoking history of more than 10 pack-years.

This study was designed as a cross-sectional, single-center, observational study. Participants were asked to fill out an asthma

control questionnaire (ACT) and underwent pulmonary function tests using an AS307 spirometer (Minato Medical Science, Osaka, Japan)²⁹ and fraction of exhaled nitric oxide (FeNO) determination using a chemiluminescence analyser (SiEVER 280i; Nippon Megacare Co., Ltd., Tokyo, Japan), in accordance with the American Thoracic Society (ATS) recommendations³⁰ and EBC sampling on the day of visiting our hospital. When medications were changed, participants with asthma were newly examined 3 months after new therapeutics were administered. Some participants with asthma were examined on and off the Japanese pollen cedar dispersal period, because the seasonal change of environment is considered as a modifier of the asthma condition in patients with asthma in real-world clinical practice.³¹ All samples were considered as obtained from different participants and counted in the analysis. Some patients with asthma and healthy participants performed sputum induction. Patients with asthma were treated with conventional medications in accordance with GINA guidelines. Medication changes were allowed at the attending physician's discretion.

This study was approved by the Institutional Review Board of Saitama Medical University Hospital (approval number: 17-114). Written informed consent was obtained from all participants.

EBC Collection

Exhaled breath condensation was collected using recommended methods with the commercially available device ECoScreen (Jaeger, Wuertzburg, Germany).³² Participants perform quiet breathing (tidal breathing) for 10 minutes without a nose clip. Samples were collected in a sterile Eppendorf tube and were immediately stored at -80°C for later analysis.

Sputum Induction and Processing

Participants performed sputum induction as described previously.³³ After premedication with salbutamol using a metered dose inhaler, sterile hypertonic saline (4.5%) was inhaled at room temperature using an ultrasonic nebuliser (MU-32; Azwell, Osaka, Japan). Sputum induction was performed at 5-minute intervals for up to 20 minutes.

Sputum samples collected in polypropylene tubes were weighed and homogenized with Hanks' balanced salt solution (1 mL) containing 1% dithiothreitol (Sigma, St. Louis, MO) at ambient temperature. After dilution with Hanks' balanced salt solution to 5 mL, samples were centrifuged at 400g for 10 minutes. The supernatant was separated and stored at -80°C for later analysis. Pellets were used for differential cell counts. Cell slides for microscopy were prepared by cytopsin (Cytospin 3; Shandon, Tokyo, Japan) and subjected to May-Grunwald-Giemsa staining. An independent investigator counted at least 500 inflammatory cells to determine their distribution in sputum. When squamous epithelial cells accounted for 50% or fewer of the total cells, samples were considered to be appropriate for analysis.^{34,35}

Measurement of EBC and Sputum Eicosanoid Levels

Exhaled breath condensate PGD2, CysLT, leukotriene B4 (LTB4), and TXB2, a nonenzymatically hydrolyzed TXA2, levels and sputum supernatant PGD2 and CysLT levels were measured using sandwich enzyme immunoassay kits (Cayman Chemicals, Ann Arbor, MI). Measurements were performed in duplicate. Because of chemical instability of PGD2, we measured PGD2-methoxime (PGD2-MOX), which was PGD2 converted by methoxylamine hydrochloride when it was measured using commercial assay kit according to the manufacturer's instructions, to prevent it from undergoing further chemical degradation. Detection lower limits for these assays were 2.0, 7.8, 3.9, and 1.6 pg/mL for PGD2-MOX, CysLTs, LTB4, and TXB2,

respectively. When any value was lower than the lower limit or not detected, it was excluded.

Measurement of Sputum Trypsin Level

The sputum supernatant trypsin level was measured using sandwich enzyme immunoassay kits (Cloud-Clone, Park Row, TX). Measurements were performed in duplicate. The lower limit of detection for these assays was 3.1 pg/mL.

Statistical Analysis

Exhaled breath condensate and sputum eicosanoid levels were log-transformed for static analysis and are described as the geometric mean (log standard error of the mean). Comparisons for parametric continuous variables were performed by Student's *t* test or analysis of variance (ANOVA), whereas those for nonparametric variables were performed by the Mann-Whitney *U* or Kruskal-Wallis test. Initial analyses for which a significant difference was identified were subjected to further testing by Bonferroni correction or the Mann-Whitney *U* test. Associations between parameters were determined using Spearman's correlation test. Significant coefficient values for associations between eicosanoids and fractional expiratory volume in 1 second (FEV₁) or forced vital capacity (FVC) were further evaluated by partial correlation analysis adjusted by age, height, and sex as control variables. Categorical variables were tested using χ^2 tests. Values are described as means $\pm$ standard deviations or medians with 25th–75th percentile ranges. Data were analyzed using SPSS version 20.0 (IBM, Armonk, NY).

Results

Participants

One hundred four EBC samples were obtained from 11 healthy participants and 93 patients with asthma. Ten EBC samples were excluded because of insufficient volume for measurement. In 89

(92.6%), 84 (89.4%), 80 (85.1%), and 91 (96.8%) EBC samples, PGD2, CysLTs, LTB4, and TXB2, respectively, were detectable. In total, 43 of 53 sputum samples obtained were appropriate for analysis (81.1%). Prostaglandin D2 and CysLT levels were above the lower limit value in all adequate sputum samples.

Characteristics of patients with asthma and healthy participants are shown in Table 1. Patients with asthma were significantly older and had lower pulmonary function. No significant differences were seen in distributions of sex, body mass index, atopy prevalence, and FeNO among patients with asthma and healthy participants.

Morbidity duration was the longest and body mass index was the lowest in the severe asthma group among all patients with asthma. Patients with severe asthma also received the highest dose of inhaled corticosteroid (ICS), and most of them took leukotriene receptor antagonist (LTRA). Some patients with severe asthma received oral corticosteroids (approximately 20%) and anti-immunoglobulin E (IgE) antibody (30%). Serum IgE levels tended to be lower in patients with severe asthma. Almost all pulmonary function parameters were the lowest in patients with severe asthma among all patients with asthma. No significant differences were found in age, sex, atopy prevalence, ACT, and FeNO among the 3 asthma severities.

EBC Eicosanoid Levels

Exhaled breath condensate PGD2-MOX and CysLT levels in EBC were significantly higher in all patients with asthma (Fig 1A, B, eTable 1). The EBC PGD-MOX levels were significantly higher in severe asthma than in moderate asthma. Exhaled breath condensate CysLT levels were also significantly higher in severe asthma than in moderate asthma and in healthy participants. The EBC LTB4 and TXB2 levels in all patients with asthma did not differ from those in the healthy participants (Fig 1C, D, eTable 1). However, EBC LTB4 levels were significantly higher in severe asthma than in moderate asthma.

Table 1
Demographics of Subjects

	Healthy control subjects	Subjects with asthma				P value	
		All	Mild	Moderate	Severe	Healthy vs all with asthma	Among 3 asthma severities
N	10	84	10	30	44		
Age, years	40.9 (13.6)	52.2 (16.8)	44.9 (13.0)	52.0 (17.1)	54.1 (17.2)	<i>P</i> < .05	NS
Female/male, n	4/6	57/27	8/2	17/13	32/12	NS	NS
BMI, kg/m ²	22.5 (22.2–22.9)	22.2 (20.3–24.4)	24.3 (22.8–29.0)	22.5 (20.4–25.4)	21.0 (19.4–24.3) ^a	NS	.02
Duration of asthma (y)	NA	9.0 (4.0–21.0)	12.0 (3.0–14.0)	4.5 (1.0–7.3)	17.0 (8.3–24.8) ^b	NA	<.0001
Atopy, n (%)	0 (0)	81 (96.4)	10 (100)	29 (96.7)	42 (95.3)	NA	NS
Medication							
ICS dose (μ g/d)	NA	550 (400–800)	200 (150–400)	400 (320–480)	800 (600–900) ^{b,c}	NA	<.0001
LABA, n (%)	NA	50 (59.5)	0 (0)	22 (73.3)	28 (63.6)	NA	.001
LTRA, n (%)	NA	68 (81.0)	4 (40.0)	20 (66.6)	43 (97.7)	NA	<.0001
Oral corticosteroids, n (%)	NA	10 (11.9)	0 (0)	0 (0)	10 (23.3)	NA	.03
Anti-IgE antibody, n (%)	NA	13 (15.5)	0 (0)	0 (0)	13 (30.2)	NA	.001
ACT	NA	23.0 (19.0–24.8)	25.0 (21.0–25.0)	24.0 (19.8–25.0)	21.0 (18.0–24.0)	NA	NS
Total log IgE, IU/L	NA	2.3 (0.4)	2.7 (1.0)	2.3 (0.5)	2.2 (0.5) ^d	NA	.03
FeNO, ppb	18.5 (8.8–29.5)	24.0 (12.0–39.8)	40.0 (30.0–75.0)	23.5 (19.5–50.8)	25.0 (17.0–31.0)	NS	NS
Basal pulmonary function							
FEV ₁ , L/min	3.3 (1.0)	2.2 (0.7)	2.4 (0.7)	2.6 (0.8)	1.9 (0.5) ^b	<.0001	<.0001
FEV ₁ , % predicted	99.3 (16.0)	87.3 (15.4)	93.9 (16.3)	94.1 (12.9)	81.0 (14.4) ^{e,f}	.02	<.0001
FVC, L/min	4.1 (1.2)	2.9 (0.7)	3.1 (0.7)	3.2 (0.8)	2.6 (0.5) ^b	<.0001	.001
FVC, % predicted	102.5 (15.7)	92.0 (11.9)	100.2 (12.5)	95.6 (10.6)	87.8 (10.7) ^{g,h}	.01	<.0001
FEV ₁ /FVC, %	82.6 (3.6)	77.1 (11.4)	77.3 (3.6)	81.3 (7.9)	74.2 (13.4) ⁱ	.002	.03

Data are expressed as mean (SD) unless otherwise indicated. Nonparametric data are expressed as median (25% to 75%).

P values were calculated using ANOVA for variables with a parametric distribution, Kruskal-Wallis test for nonparametric data, and Fisher's exact test for comparison of proportions. Initial analyses with a significant difference were further explored by post hoc pairwise analyses with Bonferroni correction.

Values were compared within the 3 asthma subgroups. vs patients with mild asthma: ^a*P* = .01; ^c*P* < .0001; ^d*P* = .02; ^e*P* = .02; ^f*P* = .004; vs patients with moderate asthma: ^b*P* < .0001; ^g*P* = .001; ^h*P* = .01; ⁱ*P* = .02.

ACT, asthma control test; ICS, inhalation corticosteroid (fluticasone), where 2 μ g beclomethasone = 2 μ g budesonide = 1 μ g fluticasone; LABA, long-acting β 2-agonist; LTRA, leukotriene receptor antagonist; NA, not applicable.

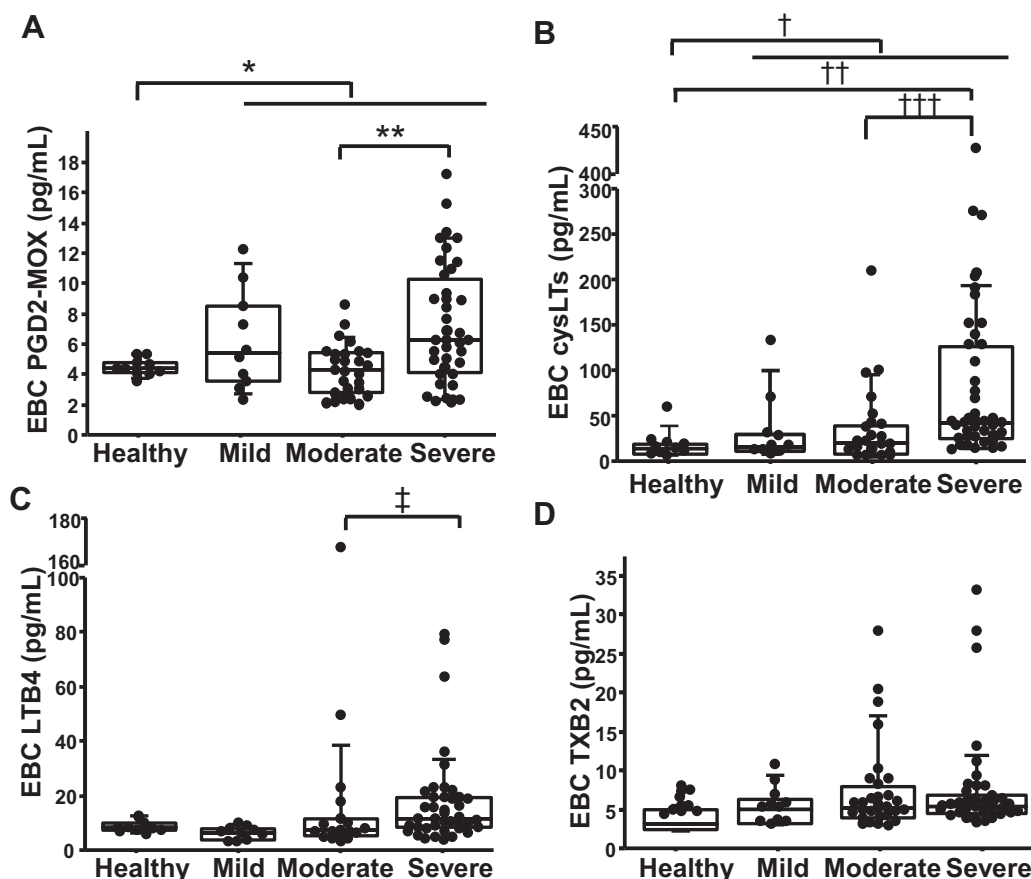


Figure 1. Exhaled breath condensate (EBC) eicosanoids in patients with asthma and healthy control participants. EBC A) prostaglandin D2-MOX (PGD2-MOX), B) cysteinyl leukotrienes (CysLTs), C) leukotriene B4 (LTB4), and D) thromboxane B2 levels in patients with asthma and healthy control participants. Values are log-transformed for statistical analysis. Log EBC PGD2-MOX levels: * $P < .05$, ** $P = .003$. Log EBC CysLT levels: † $P = .007$, †† $P = .001$, ††† $P = .016$. Log EBC LTB4 levels: ‡ $P < .0001$.

Eicosanoid Level Association in EBC with Pulmonary Function

Exhaled breath condensate PGD2-MOX levels were negatively correlated with FEV₁, FVC, and %FVC in all patients with asthma (Fig 2A, Table 2). Furthermore, partial correlation analysis indicated that EBC PGD2-MOX levels were significantly negatively correlated with FEV₁ and FVC in all patients with asthma (FEV₁, $r = -0.37$, $P = .009$; FVC, $r = -0.42$, $P = .002$). Conversely, EBC

CysLT levels were not associated with any pulmonary function parameters in patients with asthma patients (Fig 2B, Table 2). The EBC LTB4 levels were also significantly negatively correlated with %FVC in all patients with asthma (Table 2). The EBC TXB2 level was marginally correlated with FEV₁ (Table 2). However, this significant correlation was abrogated by using the partial correlation analysis ($r = 0.05$, $P > .05$). No eicosanoids were correlated with FeNO or ACT.

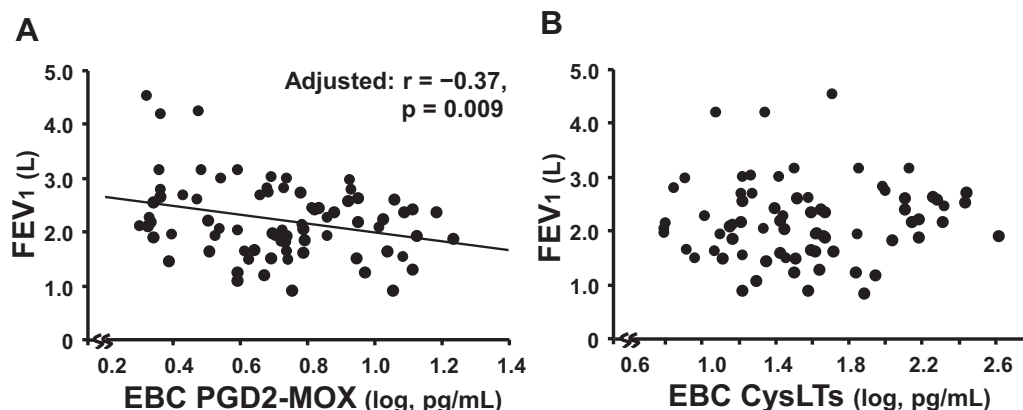


Figure 2. Relationships between EBC PGD2-MOX and CysLT levels and FEV₁ in patients with asthma. Fractional expiratory volume in 1 second is negatively correlated with A) EBC PGD2-MOX ($r = -0.24$, $P = .03$) but not B) EBC CysLT levels ($n = 83$). Fractional expiratory volume in 1 second is also negatively correlated with EBC PGD2-MOX, which is adjusted by age, height, and sex as control variables. Statistical significance of the levels was determined by Spearman's correlation test and partial correlation analysis. Exhaled breath condensate PGD2-MOX and CysLT levels are expressed after log transformation. CysLT, cysteinyl leukotriene; EBC, exhaled breath condensate; FEV₁, fractional expiratory volume in 1 second; PGD2-MOX, prostaglandin D2-methoxime.

Table 2
Correlation of Eicosanoid Levels in EBC with Pulmonary Function in Patients with Asthma

	PGD2-MOX ^a		CysLTs ^a		LTB4 ^a		TXB2 ^a	
	r	P value	r	P value	R	P value	r	P value
FEV ₁ , L/min	−0.22	<.05	0.1	NS	−0.12	NS	0.22	<.05
FEV ₁ , % predicted	−0.10	NS	−0.05	NS	−0.07	NS	0.11	NS
FVC, L/min	−0.28	.02	0.07	NS	−0.20	NS	0.15	NS
FVC, % predicted	−0.39	<.0001	0.002	NS	−0.22	<.05	−0.06	NS
FEV ₁ /FVC, %	0.0	NS	0.04	NS	0.02	NS	0.21	NS

NS, not significant.

^aValues of PGD2-MOX, CysLTs, LTB4, and TXB2 in EBC were transformed to geometric means for analysis. P values were calculated using Spearman's correlation test.

Different PGD2 and CysLT Levels in Sputum from Those in EBC in Patients with Asthma

We focused on sputum PGD2-MOX and CysLT levels because only these eicosanoids in EBC were increased in all patients with asthma. Sputum PGD2-MOX and CysLT levels were significantly higher in all patients with asthma than in healthy participants (Fig 3A, B, eTable 1), but were comparable among all asthma severities. Sputum PGD2-MOX and CysLT levels were not correlated with those in EBC in patients with asthma (Fig 3C, D, Table 3). Sputum PGD2 and CysLT levels were also not associated with pulmonary function in all patients with asthma (data not shown).

Airway Eicosanoid Association with Inflammatory Cells in Sputum

Sputum eosinophil and neutrophil ratios were increased in patients with asthma compared with those in healthy participants. Sputum eosinophil ratios were higher in mild and moderate patients with asthma than in patients with severe asthma (Table 4). Sputum tryptase level, as an alternative marker of activated mast

cells, was not increased in patients with asthma compared with that in healthy participants and did not differ among all asthma severities (Table 4).

Exhaled breath condensate PGD2-MOX levels were significantly correlated with sputum tryptase levels in all patients with asthma (Fig 4A, Table 3), which remained significant when analyzing patients with severe asthma alone ($r = 0.62$, $P < .01$), but was not in patients with mild-to-moderate asthma. The EBC PGD2-MOX levels were not associated with the proportions of eosinophils or neutrophils among all cells in sputum in all patients with asthma (Table 3). Exhaled breath condensate CysLT level was not associated with tryptase levels or the proportions of eosinophil or neutrophil among all cells in sputum in all patients with asthma (Fig 4B, Table 3). Sputum PGD2 and CysLT levels were correlated with increases in the proportions of eosinophil among all cells in sputum in all patients with asthma (PGD2: $r = 0.61$, CysLTs: $r = 0.62$, $P < .0001$ for both; Fig 4C, D). Sputum PGD2 level was not correlated with sputum tryptase level in all patients with asthma (data not shown).

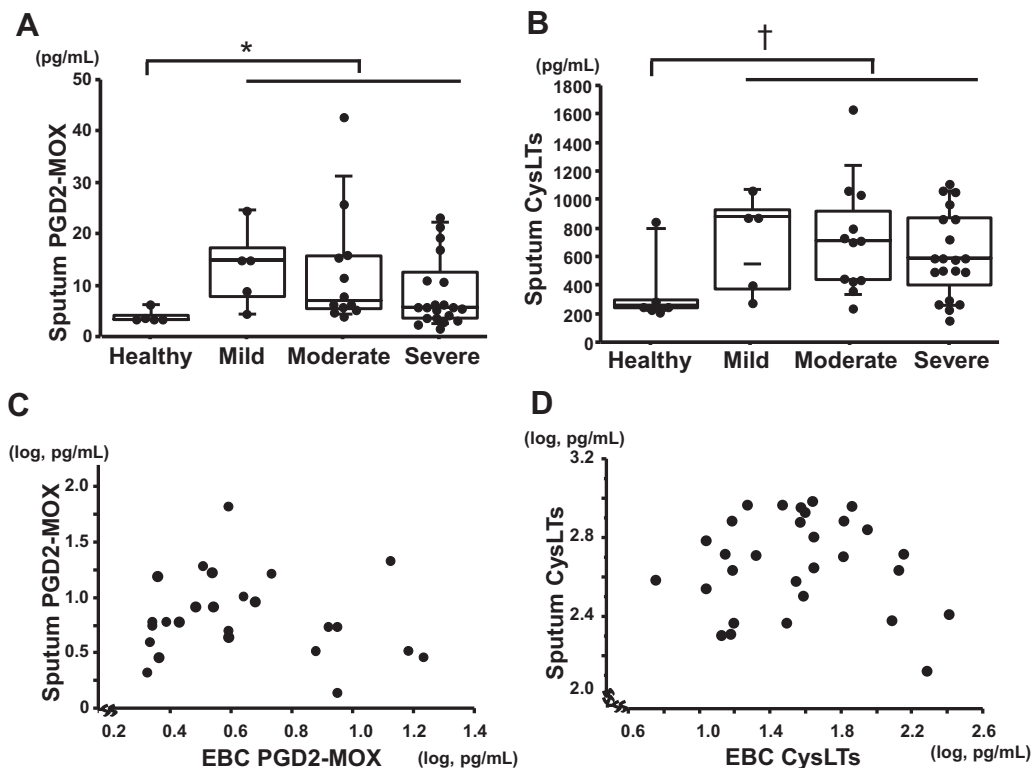


Figure 3. Sputum PGD2-MOX and CysLT levels in patients with asthma and healthy participants, and the correlations of these 2 eicosanoids between EBC and sputum in patients with asthma. Sputum A) PGD2-MOX and B) CysLT levels are significantly increased in patients with asthma ($n = 37$) compared with those in healthy participants ($n = 6$). Exhaled breath condensate C) PGD2-MOX and D) CysLT levels are not correlated with those in sputum in patients with asthma. C) and D) Values are expressed after log transformation. A) and B) * $P < .05$ and † $P < .01$ for the differences of log EBC CysLT and log EBC PGD2-MOX levels between all patients with asthma and healthy participants, respectively. CysLT, cysteinyl leukotriene; EBC, exhaled breath condensate; FEV₁, fractional expiratory volume in 1 second; PGD2-MOX, prostaglandin D2-methoxime.

Table 3

Correlation of PGD2-MOX and CysLTs Levels in EBC with Inflammatory Cell Ratios/Tryptase Levels in Sputum

	Sputum									
	PGD2-MOX		CysLTs		Tryptase		Eosinophil ratio		Neutrophil ratio	
	r	P value	r	P value	r	P value	r	P value	r	P value
EBC PGD2-MOX	−0.07	NS	−0.03	NS	0.39	.03	0.09	NS	−0.01	NS
EBC CysLTs	−0.12	NS	−0.02	NS	−0.05	NS	0.04	NS	0.07	NS

Values of PGD2-MOX and CysLTs in EBC and sputum were transformed to geometric means for analysis. *P* values were calculated using Spearman's correlation test. EBC, exhaled breath condensate; CysLT, cysteinyl leukotrienes; PGD2-MOX, prostaglandin D2-methoxime.

Discussion

This study has demonstrated the existence of similar increments of EBC and sputum PGD2-MOX and CysLTs in patients with asthma as compared with the healthy participants, and that EBC PGD2-MOX, CysLTs, and LTB4 increase in a manner associated with asthma severity. Increases in EBC PGD2-MOX exhibited a particular association with the deterioration of pulmonary function and increased sputum tryptase levels. The distinctiveness of the features of sputum PGD2-MOX and CysLTs from those in EBC also indicated that these 2 sputum eicosanoids are associated with the proportions of eosinophil in sputum, but not with asthma severity level. This indicates that sputum PGD2-MOX and CysLTs may be beneficial markers for eosinophilic airway inflammation. These results suggest that the persistent production of eicosanoids, especially PGD2, occurs regardless of the administration of current therapeutics in asthma and is involved in mast cell- and eosinophil-associated airway inflammation.

The comparison of EBC and sputum PGD2-MOX levels in stable patients with asthma with those in healthy participants demonstrated their significant increases in the former group. These increases in EBC were also amplified in patients with severe asthma. In particular, PGD2-MOX in EBC was negatively correlated with pulmonary function in patients with asthma. These are novel findings for inflammatory prostanoids in airway secretions obtained by 2 noninvasive procedures in patients with asthma, showing their clinical contribution to asthma. Several studies have shown increases of PGD2 in BALF in patients with severe asthma, but not in EBC nor in sputum.^{5–7,19–22} Our findings coincide with the results obtained in BALF, suggesting that PGD2 consistently increases in patients with asthma, especially in patients with severe asthma, regardless of whether any therapeutics including high-dose ICS or oral corticosteroids have been administered. In support of this, several studies demonstrated increases of cells expressing CRTH2, a receptor for PGD2, in poorly controlled asthma,⁶ and high expression of the CRTH2 gene on ILC2s in severe asthma.^{11,13} Prostaglandin D2 was also shown to promote the migration of human blood ILC2s and the production of type 2 cytokines by these cells,^{9,10,13} which can be enhanced by LTs.¹² Human blood ILC2s exert resistance to corticosteroids.¹⁹ Thus, PGD2 may serve as a crucial functional prostanoid in severe asthma. Recently, a CRTH2 antagonist has also attracted attention as a novel target of

treatment in asthma.^{13,36,37} Thus, the monitoring of PGD2 in the airway might be beneficial for evaluation of severe asthma.

Higher EBC PGD2 levels were associated with higher sputum tryptase levels. This result coincides with those in a previous study on the correlation of PGD2 with tryptase in BALF.⁵ That study also demonstrated that the ratio of mast cells (MCs) with tryptase and chymase staining (MCTC) to all MCs in bronchial submucosa and epithelium was higher in patients with severe asthma than in those with mild asthma, and that the patterns of increases of PGD2 in BAL appeared to parallel those of MCTC in bronchial submucosa and epithelium.⁵ Therefore, EBC PGD2 is probably delivered from activated MCs in the airway in asthma. Mast cells in the airway are difficult to detect and monitor. Exhaled breath condensate PGD2 might be a useful alternative biomarker of activated MCs, because the examination of EBC is less invasive than that for BAL and sputum induction.

Our results indicate that increases of EBC PGD2 but not CysLTs are associated with impairment of pulmonary function in asthma. This is a reasonable result because the constrictive effect of CysLTs on the bronchus was diminished by leukotriene receptor antagonist (LTRA) but that of PGD2 was not. Most of the participants with asthma (approximately 80%) in this study were treated with LTRA. These patients may have been prevented from airflow limitation by LTRA, influencing the association between CysLTs and pulmonary function in patients with asthma. Conversely, the effect of PGD2 might be preserved in patients with asthma. Our results suggest that endogenous PGD2 may continuously contribute to the deterioration of pulmonary function in asthma, despite treating patients with asthma with options including LTRA.

The CysLT levels were increased in EBC in stable patients with asthma compared with the levels in healthy participants. Exhaled breath condensate CysLT and LTB4 levels were the highest in patients with severe asthma. Our results are consistent with those in previous studies, most of which addressed childhood asthma.^{1,14,15,21,24–26} Our study confirmed that EBC CysLTs and LTB4 increased in adult asthma, suggesting that these eicosanoids are persistently produced despite the optimal treatment of asthma being administered at any age. Recent studies have shown that the biological functions of ILC2 were promoted by CysLTs,^{11,12} PGD2, and LTE4, and further enhanced by their use in combination.¹² Blood ILC2s exposed to thymic stromal lymphopoietin and ILC2s in BAL in patients with asthma were shown to be resistant to

Table 4

Comparison of Tryptase and Granulocyte Ratios in Sputum between Healthy Subjects and Patients with Asthma, and among Asthma Severity Groups

	Healthy control subjects (n = 6)		Patients with asthma				P value	
			All (n = 37)	Mild (n = 5)	Moderate (n = 12)	Severe (n = 20)	Healthy vs all patients with asthma	Among 3 asthma severities
Tryptase (pg/mL)	74.7 (48.7–110.4)		98.8 (54.9–128.5)	101.4 (33.4–110.3)	106.6 (54.9–15.9)	96.0 (63.9–230.3)	NS	NS
Eosinophils (%)	0.5 (0.0–1.3)		3.1 (0.4–9.6)	3.7 (0.5–7.5)	3.4 (2.3–11.8)	1.2 (0–10.5)	<i>P</i> < .05	NS
Neutrophils (%)	59.6 (46.7–61.2)		27.6 (13.0–41.3)	30.2 (29.3–36.9)	32.2 (15.6–59.3)	23.6 (11.4–33.5)	<i>P</i> < .01	NS

Values are presented as median (25%–75%).

P values were calculated using Mann-Whitney *U* test/Kruskal-Wallis test for nonparametric data. Overall *P* values are from comparisons among healthy control subjects and each severity of asthma.

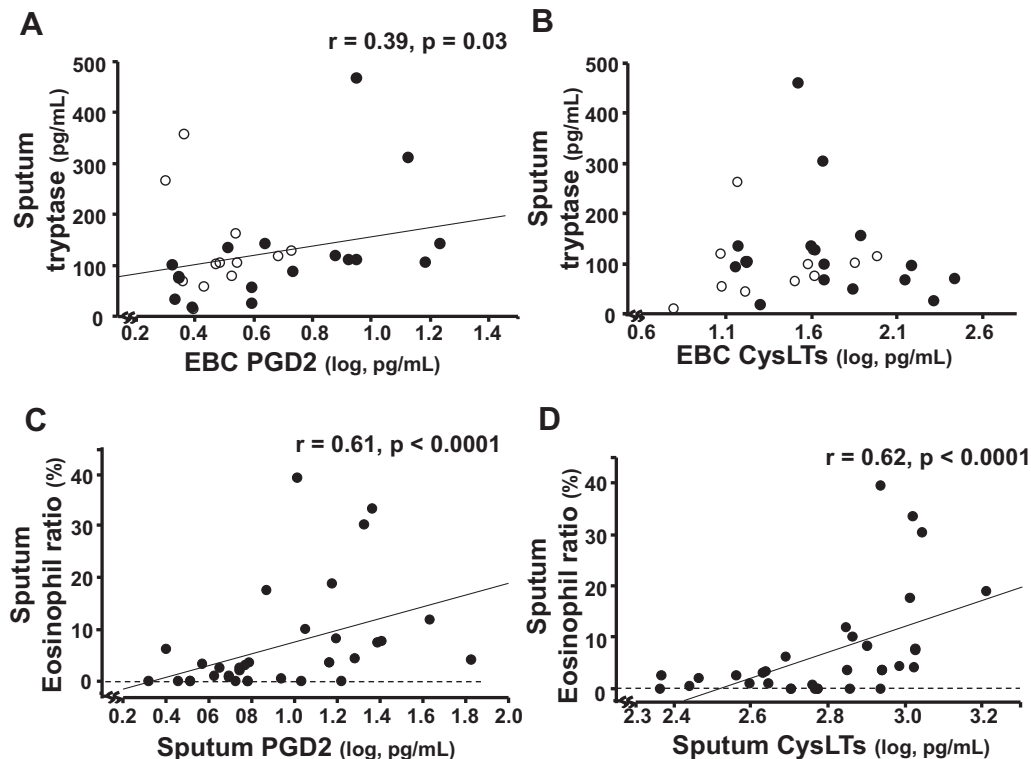


Figure 4. Correlations of EBC PGD2-MOX and CysLT levels with sputum tryptase levels/eosinophil ratio in patients with asthma. A) Exhaled breath condensate PGD2-MOX levels are significantly correlated with sputum tryptase levels in patients with asthma ($n = 38$) ($r = 0.39$, $P = .03$), which remains significant when limited to patients with severe asthma ($n = 21$) ($r = 0.62$, $P < .01$). B) Exhaled breath condensate CysLT levels are not correlated with sputum tryptase levels/eosinophil ratio in patients with asthma. C) and D) Sputum PGD2 and CysLT levels are correlated with increases in eosinophil ratios among all cells in sputum in all patients with asthma (PGD2: $r = 0.61$, CysLTs: $r = 0.62$, $P < .0001$ for both). Statistical significance of the correlations was determined by Spearman's correlation test. PGD2-MOX and CysLT levels in EBC and sputum are expressed after log transformation. CysLT, cysteinyl leukotriene; EBC, exhaled breath condensate; FEV₁, fractional expiratory volume in 1 second; PGD2, prostaglandin D₂; PGD2-MOX, prostaglandin D₂-methoxime.

dexamethasone.^{38,39} Therefore, higher CysLTs in cooperation with PGD2 might contribute to the functional status of ILC2 and resistance to corticosteroid in severe asthma.

In our study, the findings on sputum PGD2 and CysLTs indicated a different clinical aspect to the pathophysiology of asthma from those in EBC: Two EBC eicosanoids were dissociated from those in sputum and the sputum eosinophil ratio. In contrast to EBC PGD2 and CysLT level association with asthma severity, those sputum eicosanoid levels were only positively correlated with the ratio of eosinophil in sputum. Our findings of the correlations of the 2 sputum eicosanoids with the sputum eosinophil ratio are consistent with several previous studies.^{21,40} For example, a cluster analysis study showed that the asthma subtype with eosinophilia in both sputum and blood exhibited increased sputum PGD2 and CysLTs.⁴⁰ Therefore, these previous studies, coupled with ours, clinically confirm the strong connections of both PGD2 and CysLTs with eosinophilic inflammation in asthma. Sputum PGD2 and CysLTs are thus possible biomarkers of eosinophilic airway inflammation. Conversely, EBC PGD2 and CysLTs might be markers to assess asthma severity.

The reason for these discrepancies between EBC and sputum in terms of the PGD2 and CysLT levels among asthma severities remains unclear. A potential explanation is that EBC and sputum are collected from different parts of the airway. Exhaled breath condensates are statically collected by freezing expiratory breath air, which might result in the capture of some nonvolatile mediators in peripheral airways. In support of this, the observed trend in EBC PGD2 was in agreement with the increase of BAL PGD2 in severe asthma in previous studies.^{5–7} Conversely, sputum is dynamically obtained by slightly forced expectoration, which might result in it mainly including components from the central airway.

Exhaled breath condensate LTB₄ levels differed among different asthma severity levels. This finding is consistent with previous studies, in which a higher EBC LTB₄ level was detected in children and adults with moderate to severe asthma.^{14,26} Exhaled breath condensate LTB₄ levels were associated with the impairment of pulmonary function in our study, suggesting the involvement of LTB₄ in the clinical pathophysiology of asthma.

This study has limitations. First, eicosanoids were measured in airway samples obtained in different seasons, which might have influenced the results. Because eicosanoid levels fluctuate among the seasons,³¹ our adopted method might reduce the potential effect of this, but further studies of this issue are needed. Second, values below lower limitations were excluded in this study. Nevertheless, their influences seem to be marginal because those numbers are small in the 4 eicosanoids, and ratios of those are similar among healthy volunteers and patients with asthma with 3 different asthma severity levels. Third, EBC and sputum were not concentrated to measure eicosanoid levels in them and were not examined using liquid chromatography-mass spectrometry. The EBC eicosanoid levels observed in our study were similar to those reported in previous studies, which did not perform condensation of EBC samples and liquid chromatography-mass spectrometry.^{13,14,17,21,23–25} Therefore, our methods were considered acceptable.

In conclusion, integrated approaches performed by analyzing EBC and sputum inflammatory eicosanoids revealed that prostanooids and LTs persistently increased in the airways of patients with asthma regardless of whether adequate therapy was performed. These approaches probably classify distinct features of EBC and sputum eicosanoids as indicators of asthma severity and markers of airway inflammation. Exhaled breath condensate PGD2 levels are

associated with a specific protease delivered from MCs and deteriorated lung function, raising the possibility that this specific EBC prostanoid could be an alternative biomarker for concealed sustainable effects of MCs on severe asthma. Taking these findings together, EBC and sputum inflammatory eicosanoids might be targets for treating severe asthma.

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Supplementary Data

Supplementary data related to this article can be found online at <https://doi.org/10.1016/j.anai.2019.01.014>.

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Supplementary Data

eTable 1

Comparison between EBC and Sputum Eicosanoids in Healthy Participants and Patients with Asthma, and among Patients with Different Asthma Severity Levels

		Patients with asthma				P value	
		All	Mild	Moderate	Severe	Healthy vs all patients with asthma	Among 3 asthma severity levels
EBC eicosanoids							
N	10	84	10	30	44		
PGD2-MOX (log, pg/mL)	1.64 (0.58)	1.71 (0.25)	1.71 (0.24)	1.65 (0.18)	1.82 (0.26) ^a	<i>P</i> < .05	.008
CysLTs (log, pg/mL)	1.18 (0.29)	1.58 (0.44)	1.39 (0.42)	1.37 (0.48)	1.65 (0.39) ^{b,c}	.007	.001
LTB4 (log, pg/mL)	0.92 (0.10)	0.88 (0.48)	0.81 (0.18)	1.02 (0.44)	1.13 (0.33) ^d	NS	<.0001
TXB2 (log, pg/mL)	0.76 (0.10)	0.79 (0.23)	0.71 (0.21)	0.84 (0.33)	0.81 (0.24)	NS	NS
Sputum eicosanoids							
N	6	37	5	12	20		
PGD2-MOX (log, pg/mL)	1.59 (0.16)	1.95 (0.38)	2.05 (0.29)	1.96 (0.33)	1.89 (0.25)	<i>P</i> < .05	NS
CysLTs (log, pg/mL)	2.35 (0.03)	2.80 (0.21)	2.78 (0.26)	2.79 (0.24)	2.82 (0.17)	<i>P</i> < .01	NS

EBC and sputum CysLTs, LTB4, PGD2-MOX, and TXB2 levels were transformed to geometric means for analysis.

P values were calculated using Student's *t* test/1-way ANOVA for variables with a parametric distribution. Initial analyses with a significant difference were further explored by post hoc pairwise analyses with Bonferroni correction. Overall *P* values are for comparisons among healthy control participants and each asthma severity level.Vs healthy; ^b*P* = .001.Vs patients with moderate asthma: ^a*P* = .003, ^c*P* = .016, ^d*P* < .0001.

CysLT, cysteinyl leukotrienes; EBC, exhaled breath condensate; LTB4, leukotriene B4; PGD2-MOX, prostaglandin D2-methoxime; TXB2, thromboxane B2.