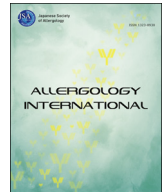




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Letter to the Editor

Eicosanoids in exhaled breath condensate of airway inflammation in patients with asthma

Dear Editor,

Asthma is a disease characterized by reversible airflow limitation with chronic airway inflammation.¹ Exhaled breath condensate (EBC) has been reported to be a useful, non-invasive method for monitoring airway inflammation.¹ This method can be used to collect aerosolized particles from airway epithelial lining fluid contained in exhaled breath and can directly measure airway biomarkers that may contribute to airway inflammation. Until now, a number of mediators had been detected in the EBC from asthmatic patients, and eicosanoids in EBC have been receiving attention as biomarkers in asthma.² Eicosanoids such as cysteinyl leukotrienes (Cys-LTs), prostaglandins (PGs), and thromboxane (TXs) induce the airway muscle constriction, airway hyper-responsiveness, increased vascular permeability, and airway hypersecretion that are thought to be involved in the airway disease of asthma.³ However, knowledge about eicosanoids in EBC is still limited. Therefore, we measured eicosanoids in EBC and analyzed according to the control status of asthma compared to healthy subjects.

The patients were recruited in Saitama Cardiovascular and Respiratory Center. The diagnosis of bronchial asthma was based on the Japanese Guidelines for Diagnosis and Management of Asthma.⁴ The patients divided into two groups according to clinical control levels of asthma and the dose of inhaled corticosteroids (ICS) prescribed. Control levels of asthma were defined as previously described⁵; in brief, the totally controlled group was defined by achievement of no symptoms, no exacerbations, no visits to the emergency room, or no medication-related adverse events. The cases that did not fulfill these criteria were defined as the not totally controlled group. Levels of doses of inhaled corticosteroids (ICS) were defined as low-dose when patients were treated with no more than 400 µg of fluticasone propionate (FP), or high-dose when treated with more than 400 µg of FP. The control group consisted of healthy subjects without a history of asthma or any other allergic disease. EBC samples were collected with a specially designed condensing chamber (EcoScreen®; Jaeger, Hoechberg, Germany) in accordance with American Thoracic Society/European Respiratory Society recommendations.¹ The measurements of EBC were conducted in the Allergy Center of the Saitama Medical University. Concentrations of thromboxane B₂ (TXB₂), leukotriene B₄ (LTB₄), prostaglandin D₂ (PGD₂), and cysteinyl leukotrienes (Cys-LTs) in EBC were measured using an enzyme-linked immunosorbent assay (ELISA) kit with a commercially available system (Cayman Chemical Company; Ann Arbor, MI, USA). Values are

expressed as means ± SEM. Two groups were compared using Student's t test and more than two groups were compared using a repeated-measures analysis of variance (ANOVA) with Dunn's multiple comparison test. Values of $p < 0.05$ were considered significant.

Twenty-eight patients with asthma were divided into a totally controlled ($n = 15$) group and a not totally controlled ($n = 13$) group according to the status of asthma control at the time of sampling.⁵ Healthy subjects ($n = 11$) were established, and the groups were then compared. Additionally, patients with asthma were divided into an untreated group (naïve, $n = 8$) and a group treated with ICS ($n = 20$). The ICS-treated group was further divided into a group treated with low-dose ICS ($n = 13$) and a group treated with high-dose ICS ($n = 7$), and they were once again compared. Table 1 shows the baseline characteristics for each group. TXB₂ could not be measured in EBC from any of the healthy subjects. In contrast, TXB₂ was detected in 16 of the 28 patients with asthma (15.7 ± 2.0 pg/mL, 95%CI, 11.5–20.0 pg/mL, Fig. 1A), and was significantly increased with poorer control subjects (totally controlled vs. not totally controlled: 12.9 ± 1.8 pg/mL, 95%CI, 8.0–17.8 pg/mL, $n = 5$ vs. 17.0 ± 2.7 pg/mL, 95%CI, 11.0–23.0 pg/mL, $n = 11$, $p < 0.01$, Fig. 1B). TXB₂ was also increased in the high-dose ICS group (steroid-naïve vs. low-dose ICS vs. high-dose ICS: 11.5 ± 1.4 pg/mL, 95%CI, 7.5–15.6 pg/mL, $n = 5$ vs. 15.0 ± 2.8 pg/mL, 95%CI, 7.7–22.0 pg/mL, $n = 6$ vs. 20.9 ± 4.6 pg/mL, 95%CI, 8.1–33.7 pg/mL, $n = 5$, $p < 0.01$, Fig. 1C). The levels of LTB₄ were increased in untreated patients with asthma (healthy subjects vs. steroid-naïve asthma: 5.4 ± 0.7 pg/mL, 95%CI, 3.6–7.3 pg/mL, $n = 5$ vs. 5.7 ± 0.7 pg/mL, 95%CI, 3.9–7.5 pg/mL, $n = 6$, $p < 0.05$), but not in ICS-treated patients with asthma (data not shown). There was no significant difference regardless of control status (data not shown). PGD₂ were not different between patients with asthma and healthy subjects (data not shown). Cys-LTs were detected

Table 1
Clinical characteristics of the study subjects.

	Healthy subjects	Patients with asthma			p value
		Steroid-naïve	Low-dose ICS	High-dose ICS	
No.	11	8	13	7	
Male/female	7/4	4/4	8/5	1/6	0.177
Age (y)	37 ± 2	57 ± 5	51 ± 4	60 ± 4	<0.001
Allergic, no.	7	4	8	5	0.863
FEV ₁ (% predicted)	99.8 ± 2.6	93.0 ± 7.0	81.2 ± 5.9	78.8 ± 10.2	<0.05

ICS, inhaled corticosteroid; FEV₁, forced expiratory volume 1.0 s.

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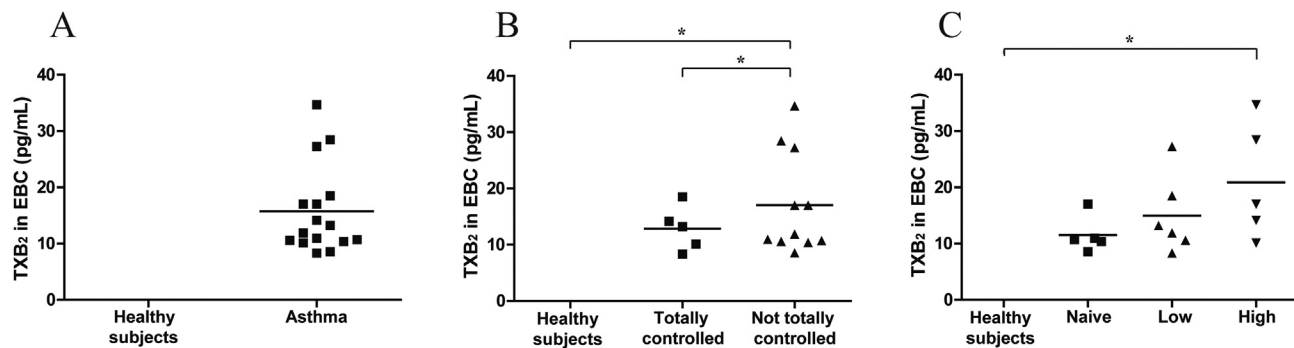


Fig. 1. Concentrations of thromboxane B₂ (TXB₂) in EBC in healthy subjects and patients with asthma. (A) TXB₂ is undetectable in healthy subjects while measurable in 16 patients with asthma. (B) Patients with asthma were divided into a totally controlled group and a not totally controlled group. (C) Patients with asthma were divided into an untreated group and an inhaled corticosteroid-treated group, with no more than 400 µg of fluticasone propionate (FP) (low-dose) and a group treated with more than 400 µg of FP (high-dose). Mean values are shown with horizontal bars. **p* < 0.01.

only in 3 patients with not totally controlled asthma who had been treated with high-dose ICS (data not shown). The relationship between eicosanoids in EBC and respiratory function was also examined, but no significant correlation was seen (data not shown).

We confirmed that TXB₂ could be measured in EBC obtained from patients with asthma. TXB₂ was increased according to disease instability, and not affected by ICS. TXB₂ is a stable metabolic product of thromboxane A₂ (TXA₂). TXA₂ is a potent bronchoconstrictor and also causes airway smooth muscle hyperplasia, a characteristic feature of asthmatic airways, *in vitro*.⁶ A metabolic product of TXB₂, 11-dehydro TXB₂ (11-DHTXB₂), was reported to be increased during exacerbation of asthma attacks and decreases when symptoms recede.⁷ In a study using EBC by Montuschi *et al.*, problems in the detection rate of TXB₂ in patients with asthma were revealed,⁸ whereas in a study by Huzar *et al.*, TXB₂ was detected in approximately 75% of EBC samples in exercise-induced asthma cases.⁹ In the present study, it was demonstrated that TXB₂ in EBC reflected asthma instability and was not modified by use of ICS. The present study showed the dynamics of TXB₂ in asthmatic airways more thoroughly than previous reports, but ethnic differences may also need to be taken into account. The usefulness of biosynthesis inhibitors and receptor antagonists for TXA₂ has been established in asthma treatment in Japan.¹⁰ However, the effect of TXA₂ inhibitors in asthma has not really been confirmed in western countries because no statistically significant difference was observed.¹¹ Therefore, it is possible that TXB₂ in EBC might be a good disease marker of asthma only in a certain ethnic group such as Japanese population.

In conclusion, while this study only targeted a small number of Japanese subjects, the present results suggest that TXB₂ in EBC may be a useful indicator for assessing the disease control or severity of asthma.

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The Institutional Review Board of the each hospital approved this study, and the written informed consent was obtained in every patients. We do not receive any financial support.

Conflict of interest

The authors have no conflict of interest to declare.

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