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Allergy International

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Original Article

Eicosanoids seasonally impact pulmonary function in asthmatic patients with Japanese cedar pollinosis

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ARTICLE INFO

Article history:

Received 29 August 2019

Received in revised form

21 March 2020

Accepted 4 April 2020

Available online xxx

Keywords:

Allergic asthma

Cysteinyl leukotrienes

Exhaled breath condensate

Japanese cedar pollen

%FEV₁

List of Abbreviations:

ACT, asthma control test; AR, allergic rhinitis; ATS, American Thoracic Society; BA, bronchial asthma; BA-AR-non-JCP, asthmatics with concomitant AR non-sensitized to JCP; BALF, bronchoalveolar lavage fluid; BA-SAR-JCP, asthmatics with concomitant SAR sensitized to JCP; CysLT, cysteinyl leukotriene; CysLT₁R, CysLT receptor type 1; CysLT₂R, CysLT receptor type 2; CysLT₃R, CysLT receptor type 3; EBC, exhaled breath condensate; FeNO, fraction of exhaled nitric oxide; FEV₁, forced expiratory volume in one second; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; JCP, Japanese cedar pollen; JCP season, JCP scattering season; LTB₄, leukotriene B₄; LTRA, leukotriene receptor antagonist; NCS, nasal corticosteroid; non-JCP season, non-JCP scattering season; OCS, oral corticosteroid; PGD₂, prostaglandin D₂; PGD₂-MOX, PGD₂-methoxime; SAR, seasonal allergic rhinitis; TXB₂, thromboxane B₂

ABSTRACT

Background: Condition of asthma in patients with asthma and concomitant seasonal allergic rhinitis (SAR) deteriorates during the Japanese cedar pollen (JCP) season. However, the underlying mechanisms remain unclear.

Methods: We analyzed seasonal variations in eicosanoid levels in the airways of patients with asthma and concomitant SAR sensitized to JCP (N = 29, BA-SAR-JCP group) and those not sensitized (N = 13, BA-AR-non-JCP group) during the JCP season. The association between changes in eicosanoid concentrations and pulmonary function was assessed. Exhaled breath condensate (EBC) was collected, and pulmonary function tests were performed during the JCP and non-JCP seasons. The cysteinyl leukotriene (CysLT), thromboxane B₂ (TXB₂), prostaglandin D₂-methoxime (PGD₂-MOX), and leukotriene B₄ (LTB₄) levels in the collected EBC were measured via enzyme-linked immunosorbent immunoassays.

Results: The log CysLT levels significantly increased in the BA-SAR-JCP group during the JCP season compared with the non-JCP season (1.78 ± 0.55 , 1.39 ± 0.63 pg/mL, mean $\pm$ standard deviation, respectively, $p = 0.01$) and those in the BA-AR-non-JCP group during the JCP season (1.39 ± 0.38 pg/mL, $p = 0.04$). Moreover, the log TXB₂ levels seemed to increase. However, the log LTB₄ and log PGD₂-MOX levels did not increase. The changes in the log CysLT levels during the two seasons were negatively correlated to forced expiratory volume in one second (FEV₁) in the BA-SAR-JCP group ($r = -0.52$, $p < 0.01$).

Conclusions: In the BA-SAR-JCP group, seasonal increases in eicosanoid levels in the airway likely promoted deterioration in pulmonary function despite optimal maintenance treatment.

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Peer review under responsibility of Japanese Society of Allergy.

<https://doi.org/10.1016/j.alit.2020.04.014>

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Introduction

Asthma deteriorates due to risk factors such as exposure to airborne allergens and viral infections.¹ A recent study has demonstrated that periods of high prevalence of asthma exacerbation are dependent on both the type of ambient allergen and the presence or absence of sensitization to a causative allergen.² Additionally, the coexistence of allergic rhinitis (AR) has been revealed to further exacerbate asthma.³ Several previous epidemiologic studies have reported that insufficient treatment of rhinitis led to asthma control deterioration in a large population of patients with asthma complicated with AR.^{4,5} In patients with asthma with concomitant AR, airway hyperresponsiveness and bronchial inflammation are seasonally enhanced, and the fraction of exhaled nitric oxide (FeNO) is increased.^{3,6–9}

Japanese cedar pollen (JCP) is a frequently occurring allergen found in Japan that causes Japanese cedar pollinosis.^{10,11} The nationwide spread of Sugi trees (*Cryptomeria*, a monotypic genus of conifer in the cypress family Cupressaceae) as well as seasonal increases in the average temperature and sunlight have gradually increased the cumulative levels of airborne JCP, which peak from March to April on Honshu, the main island of Japan, including the capital city of Tokyo as well as Saitama and other prefectures around Tokyo.^{10–12} Such environmental changes over the last two decades have significantly increased the prevalence of Japanese cedar pollinosis, which is now observed in approximately 30% of the Japanese population.^{10,13} The results of an epidemiological survey conducted in Japan have revealed that approximately 70% of patients with asthma have concomitant seasonal AR (SAR).¹⁴ In patients with asthma and comorbid SAR, the period of high levels of JCP dispersal (i.e., JCP season) aggravates the control of asthma symptoms and peak expiratory flow rate.^{11,15,16} Additionally, allergen provocation tests have demonstrated that JCP itself exerts bronchial constriction.¹⁷ However, seasonal variations in the physiological and inflammatory conditions in the airway remain to be elucidated in patients with asthma and concomitant SAR sensitized to JCP (BA-SAR-JCP). Moreover, few studies have investigated the mechanisms underlying seasonally impaired asthma control.

Eicosanoids are commonly recognized as crucial mediators of both asthma and AR. Leukotrienes and prostanoids are involved in the progression of allergic inflammation of the upper and lower airways, production of nasal polyps, direct bronchial smooth muscle constriction, and remodeling process in lower airways, resulting in persistent airway limitation.^{13,18} Cysteinyl leukotrienes (CysLTs), leukotriene B₄ (LTB₄), and prostaglandin D₂ (PGD₂) are increased in exhaled breath condensate (EBC), sputum, and bronchoalveolar lavage fluid (BALF) and have been associated with pulmonary function in asthma.^{19–24} These eicosanoids, as well as thromboxane B₂ (TXB₂), have been shown to be increased in liquid samples from the airways of patients with asthma following airway provocation with a causative allergen.^{25–29} Only one study has demonstrated an increase in CysLTs in the sputum of patients with asthma with AR by natural allergen exposure during the pollen-scattering season.⁸ However, there have been no reports regarding changes in any eicosanoids in the EBC during the pollen season. Thus, we hypothesized that eicosanoids seasonally change in BA-SAR-JCP patients.

The present study aims to evaluate the eicosanoid levels in EBC and pulmonary function during the JCP and non-JCP seasons in BA-SAR-JCP patients compared with BA-AR-non-JCP patients. The study also intends to analyze the association between EBC eicosanoids and both pulmonary function and FeNO to examine the

effects of seasonal modifications of those biomarkers on airway states in BA-SAR-JCP compared with BA-AR-non-JCP patients.

Methods

Patients

Patients with asthma and concomitant AR aged 20 years and above were recruited from the Allergy Center of Saitama Medical University Hospital. The diagnosis of asthma was made according to the Global Initiative for Asthma (GINA) guidelines as follows: clinical history of asthma symptoms and either an increase in the baseline forced expiratory volume in one second (FEV₁) of $\geq 12\%$ over the baseline values after the inhalation of 200 μg of salbutamol aerosol or the presence of bronchial hyperresponsiveness as defined by the amount of methacholine required to decrease the FEV₁ by 20% ($< 4 \text{ mg/mL}$).¹ All participants received maintenance medications for at least six months in accordance with the GINA guidelines. Outpatients with asthma were classified as having mild to moderate (steps 1–4) or severe asthma (step 5) according to the GINA guidelines. The exclusion criteria included asthma exacerbation following three or more courses of oral corticosteroids, hospitalization during the month prior to the entry period, respiratory infection during the previous month, present smoking habit, and the presence of a comorbid disease such as cancer, severe renal failure, or severe heart failure. The diagnosis of AR was made based on the guidelines of the Japanese Society of Allergy considering symptoms of AR, nasal physical examination results, and serum allergen-specific immunoglobulin E (IgE) concentrations.³⁰ A severe acute exacerbation was defined by one of the following criteria: administration of systemic corticosteroids for at least three days, an increase from a stable maintenance dose, or hospitalization or an emergency room visit for asthma requiring a systemic corticosteroid. Serum allergen-specific IgE levels were measured using the ImmunoCAP system (Pharmacia Diagnostics AB, Uppsala, Sweden) to confirm atopy after testing using the following common inhalant allergens: house dust mites, JCP, cypress, ragweed, cocksfoot, dog, cat, and airborne spores of common genera of fungi (*Aspergillus*, *Alternaria*, and *Candida*). Serum allergen-specific IgE levels $> 0.7 \text{ UA/mL}$ were considered positive.

The cohort of this cross-sectional study was limited to eligible patients examined at the Outpatient Department of Saitama Medical University Hospital during the JCP (between February and May) and non-JCP (between October and December) seasons in Japan.

The study protocol was approved by the Institutional Review Board of Saitama Medical University Hospital (approval no. 788, 789), and written informed consent was obtained from all participants.

Collection of clinical samples

Each participant underwent spirometry with an AS307 spirometer (Minato Medical Science, Osaka, Japan).³¹ Blood and EBC samples were collected, the latter of which were obtained using the recommended methods along with commercially available devices (ECoScreen; Jäger Rechtsanwalt, Würzburg, Germany) according to the American Thoracic Society (ATS)/European Respiratory Society guidelines.³² Participants tidally breathed through a mouthpiece connected to a condenser for 15 min while wearing a nose clip. The samples were immediately stored in sterile 2-mL Eppendorf tubes at -80°C for further analysis to minimize eicosanoid loss. The eicosanoids were measured approximately one year after the sample collection.

Measurement of FeNO

The FeNO was measured via a chemiluminescence analyzer (NIOX MINO; Aerocrine, Solna, Sweden) according to the recommendations of the ATS.³³

Measurement of eicosanoid concentrations in EBC

The EBC samples were analyzed in duplicate for CysLT, LTB₄, PGD₂, and TXB₂ using commercially available sandwich enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturers' instructions. However, PGD₂-methoxime (PGD₂-MOX), i.e., PGD₂ converted by methoxyamine hydrochloride, was measured instead of PGD₂ to prevent further chemical degradation due to chemical instability. The lower limits of detection for the assays were 7.8 pg/mL for CysLTs, 3.9 pg/mL for LTB₄, 2.0 pg/mL for PGD₂-MOX, and 1.6 pg/mL for TXB₂.

Airborne JCP count

Data regarding the monthly amount of airborne JCP during the JCP season were obtained from online information disclosed by the Ministry of Environment, Government of Japan.³⁴ The observation system measuring the airborne JCP levels was located in Hanno, near where the participants lived.

Statistical analysis

The values of the eicosanoid levels in the EBC, IgE, and FeNO were log-transformed for statistical analysis. The log-transformed lower limit values of them, i.e., 0.89 for CysLT, 0.59 for LTB₄, 0.3 for PGD₂-MOX, and 0.2 for TXB₂, were used when values were lower than the lower limit. Then, Kolmogorov–Smirnov or Leven tests were performed to determine the normality of the values. The Student's *t*-test was used for comparisons of parametric continuous variables of the two patient groups, while the Mann–Whitney *U* test was used for comparisons of nonparametric variables.

Table 1
Patient characteristics.

	JCP-SAR (n = 29)	Non-JCP-AR (n = 13)	p-value
Age, y, mean (SD)	49.7 (17.2)	56.7 (14.7)	n.s.
Female/male, n)	22/7	11/2	n.s.
Smokers, n (%)	1 (3.4)	0 (0)	n.s.
Duration of asthma [†] , y)	6.0 (3.0–12.0)	17.0 (12.0–22.0)	n.s.
Sensitized allergen, mite, n (%)	18 (62.1)	6 (46.2)	n.s.
Other allergens	10 (34.5)	7 (53.8)	n.s.
GINA, n (%)			
steps 1–2	2 (7.1)	3 (24.1)	n.s.
steps 3–4	12 (41.4)	2 (15.4)	n.s.
step 5	15 (51.7)	8 (61.5)	n.s.
ICS dose, µg/dL, mean (SD)	1227 (607)	1416 (895)	n.s.
Leukotriene receptor antagonist, n (%)	24 (82.8)	9 (69.2)	n.s.
Oral corticosteroid, n (%)	3 (10.3)	4 (30.8)	n.s.
NCS, n (%)	14 (48.3)	5 (38.5)	n.s.
H ₁ receptor antagonist, n (%)	12 (41.4)	7 (51.8)	n.s.
Log IgE, IU/L, mean (SD)	2.4 (0.6)	1.9 (0.4)	0.02
%FEV ₁ , %, mean (SD)	86.9 (14.8)	92.5 (15.9)	n.s.
FEV ₁ /FVC, %, mean (SD)	78.2 (10.2)	77.7 (9.1)	n.s.
FeNO [†] , ppb	24 (10–40)	30 (14–55)	n.s.

FeNO, fraction of exhaled nitric oxide; %FEV₁, % of predicted FEV₁; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; JCP-SAR, patients with asthma with seasonal allergic rhinitis sensitized to Japanese cedar pollen; non-JCP-AR, patients with asthma with allergic rhinitis not sensitized to Japanese cedar pollen; NCS, nasal corticosteroid; n.s., nonsignificant; SD, standard deviation.

[†] Expressed as median (interquartile range). ICS is expressed as budesonide equivalent.

Associations between the eicosanoids and clinical indices were analyzed using the Spearman correlation test. Categorical variables were analyzed using the chi-squared test. The data were presented as the mean ± standard deviation or median with 25th to 75th percentile ranges if not normally distributed and analyzed using the SPSS software version 20.0 (IBM, Armonk, New York, NY, USA).

Results

Characteristics of patients with asthma

Of the 42 eligible patients, 29 and 13 patients were classified into the BA-SAR-JCP and BA-AR-non-JCP groups, respectively (Table 1). No significant differences were observed between the two groups regarding age, sex, duration of asthma, status of sensitization to allergens other than JCP, GINA treatment step, dose of ICS, ratio of LTRA and oral corticosteroid use, ratio of nasal corticosteroid and H₁ receptor antagonist use, ratio of patients with acute exacerbations during the JCP season, blood eosinophil number, pulmonary function, or log FeNO. However, the total IgE was higher in the BA-SAR-JCP group than in the BA-AR-non-JCP group. The prevalence of severe asthma was approximately 60% in both groups. Over 70% of the patients in both groups were treated with a LTRA, while less than 50% were treated using any medication for AR.

The monthly airborne JCP count increased during the JCP season from 2010 to 2013 (Fig. 1). Of the participants, 7 (24%) in 2010, 7 (24%) in 2011, and 15 (52%) in 2012 in the BA-SAR-JCP group and 7 (50%) in 2010, 6 (43%) in 2012, and 1 (7%) in 2013 were examined during the JCP season.

Eicosanoids in EBC in the BA-SAR-JCP group during the JCP season

The log CysLT and log TXB₂ concentrations in the EBC were significantly high during the JCP season in the BA-SAR-JCP group compared with the BA-AR-non-JCP group (Fig. 2a, b; Supplementary Table 1). However, the log LTB₄ and log PGD₂-MOX values did not reveal any significant increase (Fig. 2c, d; Supplementary Table 1). Additionally, the CysLT concentrations in the EBC significantly increased from the non-JCP season to the JCP season in the BA-SAR-JCP group but not in the BA-AR-non-JCP group. The log TXB₂ concentrations in the EBC also increased from the non-JCP season to the JCP season in the BA-SAR-JCP group, though not significantly. No significant differences were observed in the log LTB₄ or log PGD₂-MOX concentrations in the EBC between the two seasons in either group. In the BA-SAR-JCP group, eight log

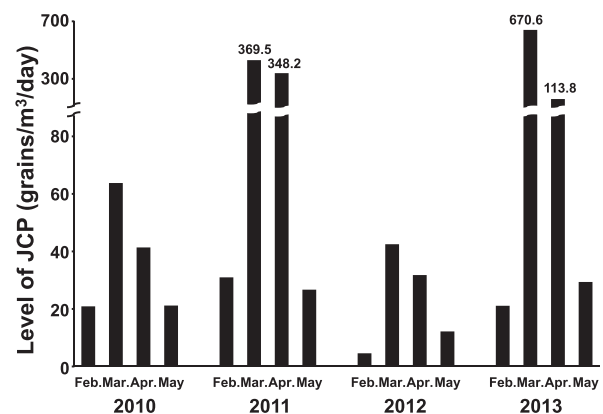


Fig. 1. Monthly Japanese cedar pollen (JCP) dispersion during the JCP season between 2010 and 2013.

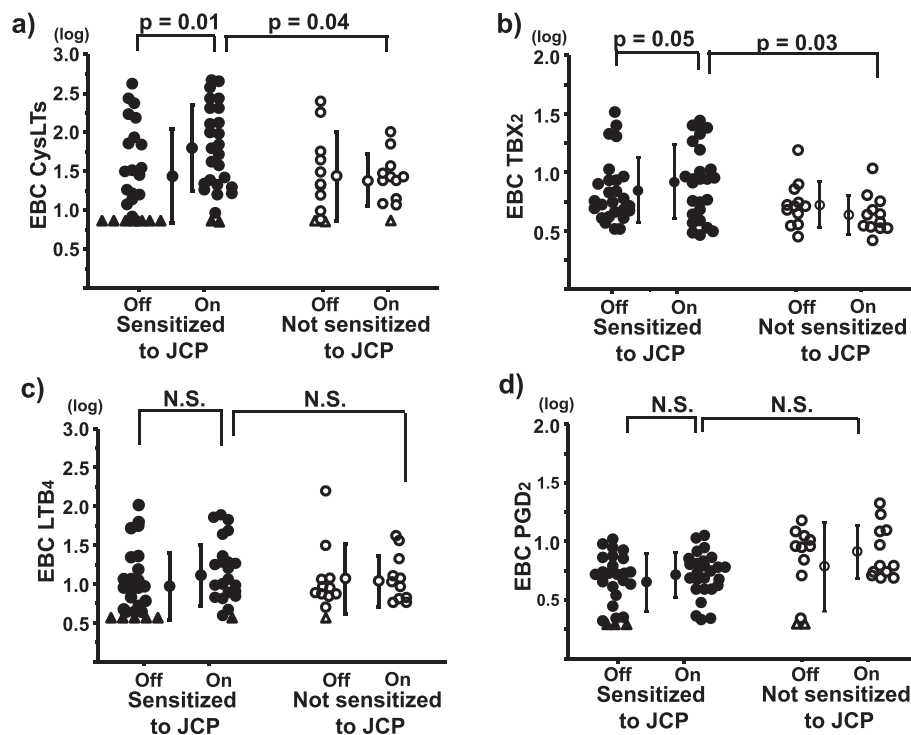


Fig. 2. Concentrations of CysLTs (a), TXB₂ (b), LTB₄ (c), and PGD₂ (d) in EBC samples during the JCP and non-JCP seasons in the BA-SAR-JCP and BA-AR-non-JCP groups. Solid circles and triangles, participants in the BA-SAR-JCP group; open circles and triangles, participants in the BA-SAR-non-JCP group; circles, participants with samples over the lower limit values; triangles, participants with samples below the lower limit values. The lower limit values for the eicosanoid EBC levels (pg/mL) are used when those levels are below the lower limit. Values have been expressed as the log-transformed for statistical analysis. EBC, exhaled breath condensate; JCP, Japanese cedar pollen; CysLTs, cysteinyl leukotrienes; TXB₂, thromboxane B₂; LTB₄, leukotriene B₄; PGD₂, prostaglandin D₂; N. S., not significant.

CysLT, five log LTB₄, and three log PGD₂-MOX measurements during the non-JCP season and two log CysLT and one log LTB₄ measurements during the JCP season were below the lower limit value. In the BA-AR-non-JCP group, two log CysLT, one log LTB₄, and two log PGD₂-MOX measurements during the non-JCP season and one log CysLT during the JCP season were below the lower limit value.

Pulmonary function and FeNO in the BA-SAR-JCP group during the JCP season

No pulmonary function parameter changed from the non-JCP season to the JCP season in either the BA-SAR-JCP or BA-AR-non-JCP group (Table 2). In addition, the two groups did not differ in terms of pulmonary function parameters during the JCP season (Table 2).

No significant differences were observed in the log FeNO levels between the two patient groups during the JCP season (Table 2), and no significant increases were observed from the non-JCP season to the JCP season in either group (Table 2).

Correlation between the changes in EBC eicosanoid concentration and deterioration of pulmonary function

Changes in the log CysLT levels were significantly inversely correlated with the changes in FEV₁, %FEV₁, forced vital capacity (FVC), and FEV₁/FVC in the BA-SAR-JCP group but not in the BA-AR-non-JCP group (Fig. 3a; Table 3). Changes in the log TXB₂ EBC levels also tended to be inversely correlated with the changes in the FEV₁ and significantly correlated with the %FEV₁ and FEV₁/FVC in the BA-SAR-JCP group (Fig. 3b; Table 3). A similar association was observed for the changes in the log LTB₄ EBC levels with those in the %FEV₁ (not significant) and FEV₁/FVC ($p < 0.02$) (Fig. 3c; Table 3). Changes

Table 2

Comparison of pulmonary function and FeNO during the on-JCP and off-JCP seasons between the BA-SAR-JCP and BA-AR-non-JCP groups.

	SAR-JCP (n = 29)			AR-non-JCP (n = 13)		
	Off-pollen season	On-pollen season	p-value	Off-pollen season	On-pollen season	p-value
FEV ₁ , L	2.5 (0.8)	2.4 (0.6)	n.s.	1.8 (0.6)	1.9 (0.5)	n.s.
%FEV ₁ , %	88.2 (17.3)	90.3 (13.7)	n.s.	84.3 (9.4)	88.9 (17.9)	n.s.
FVC, L	3.1 (0.7)	3.0 (0.6)	n.s.	2.3 (0.5)	2.5 (0.6)	n.s.
%FVC, %	94.8 (9.7)	94.3 (10.6)	n.s.	88.0 (14.1)	95.6 (15.2)	n.s.
FEV ₁ /FVC, %	79.3 (11.5)	81.2 (10.6)	n.s.	80.6 (10.7)	75.7 (16.1)	n.s.
Log FeNO, ppb	1.28 ± 0.40	1.35 ± 0.34	n.s.	1.46 ± 0.43	1.50 ± 0.38	n.s.

SAR-JCP, patients with asthma and seasonal allergic rhinitis sensitized to Japanese cedar pollen; AR-non-JCP, patients with asthma and allergic rhinitis not sensitized to Japanese cedar pollen; %FEV₁, percent of predicted FEV₁; %FVC, percent of predicted FVC.

in the log PGD₂-MOX levels were significantly negatively correlated with changes in the FEV₁/FVC but not with changes in the %FEV₁ in the BA-SAR-JCP group (Fig. 3d; Table 3). No correlation was observed between the eicosanoids and pulmonary function or log FeNO.

Discussion

The results of the present study demonstrated that EBC CysLTs significantly increase during the pollen season in BA-SAR-JCP patients and that the seasonal increase in EBC CysLTs is specifically correlated with decreased pulmonary function. These findings suggest that seasonal exposure to cyclically fluctuating aero-allergens causes acceleration of T helper 2 (Th2)-type airway

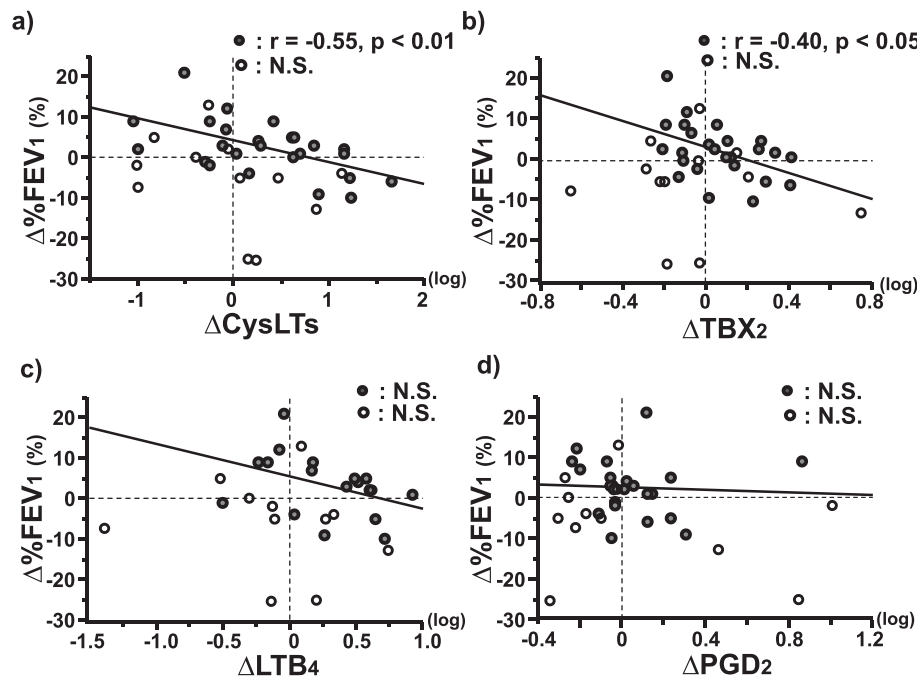


Fig. 3. Correlation between changes in %FEV₁ and CysLT (a), LTB₄ (b), PGD₂ (c), and TXB₂ levels (d) in EBC in participants with asthma. Solid circles, BA-SAR-JCP group; open circles, BA-AR-non-JCP group; solid lines, participants sensitized to JCP. The x-axes indicate the eicosanoid EBC levels (pg/mL) with logarithmic scales. EBC, exhaled breath condensate; %FEV₁, forced expiratory volume in one second of percentage predicted; CysLTs, cysteinyl leukotrienes; TXB₂, thromboxane B₂; LTB₄, leukotriene B₄; PGD₂, prostaglandin D₂; N. S., not significant.

inflammation, leading to pulmonary function deterioration. Considering the higher frequency of asthma exacerbation in the pollen dispersal season in Europe and North America,² these pathophysiological modifications of the airway appear to serve as an underlying precursor of asthma aggravation, even in well-controlled asthma. Previous studies have reported periodical worsening in terms of inflammatory cells,⁷ inflammatory mediators, bronchial hyperresponsiveness,⁷ peak expiratory flow rate,¹¹ and symptoms^{15,16} during aeroallergen scattering seasons; however, no correlation between these variables has been elucidated yet. Thus, the mechanisms underlying the clinical instability remain unknown for the pollen season. The present study addressed the involvement of certain eicosanoids in the modification of pulmonary function in BA-SAR-JCP patients during the JCP season.

In the present study, the seasonal surge in EBC CysLTs was stronger in the BA-SAR-JCP group than in the BA-AR-non-JCP group during the JCP season. This finding supports that of Tufvesson *et al.*, who reported that sputum CysLT levels increase during the pollen scattering season in mild to moderate ICS-naïve patients with asthma.⁸ In addition, our study showed seasonal CysLT increases in the airway during the JCP season via a less invasive examination of the EBC rather than sputum induction. Our results also validated that CysLT levels in the airways increase in JCP-sensitized patients with asthma and concomitant SAR during the JCP season despite appropriate treatment.

Our study validated that the amount of airborne JCP increases during the annual JCP season (Fig. 1). During the same period, a seasonal increment of EBC CysLT levels in the BA-SAR-JCP group was identified in our study, which was similar to a previous study.⁸ Moreover, another previous study demonstrated that specific interleukin-4-producing Th2 cell levels of JCP increase during the pollen season in patients with JC pollinosis, leading to the progression of Th2 cascade and the activation of mast cells and

eosinophils, which are the main sources of CysLTs.³⁵ In addition, several other studies reported that the increase in CysLT levels observed in liquid samples collected from the surface of the airway duct was caused by segmental exposure of the bronchi in humans.^{25–29} Thus, a seasonal increase in CysLT levels may be induced by natural exposure to JCP during the annual JCP season.

In this study, seasonal variations in the CysLT levels were significantly associated with changes in pulmonary function in the BA-SAR-JCP group but not in the BA-AR-non-JCP group. This detrimental relationship between CysLT levels and airway narrowing is compelling because CysLTs can cause bronchoconstriction and can progress to extensive bronchial inflammation.¹⁸ The mechanism of this finding in patients with asthma treated with LTRA remains unclear. One explanation could be that an excessive level of CysLTs and other eicosanoids or cytokines in the airway, which is higher than the amount of CysLT receptor type 1 (CysLT₁R) regulated by LTRA, energizes immune cells during the JCP season. A previous study showed that the decrease in biological function in group 2 innate lymphoid cells caused by montelukast, an LTRA, is modest in the presence of PGD₂.³⁶ The Th2 cytokines produced by these cells might activate effector cells despite treatment with LTRA, leading to decreased pulmonary function. Another explanation might be that bronchial inflammation dependent on CysLT₂R, CysLT₃R (G-protein-coupled receptor 99), or P2Y₁₂ receptors for which LTRAs have low affinity enhances airway narrowing.³⁷

In our study, seasonal fluctuations in other eicosanoids were also observed. The EBC TXB₂ levels increased in the BA-SAR-JCP group, whereas the EBC LTB₄ and PGD₂-MOX levels did not, suggesting that eosinophils are primarily involved in the amplified production of CysLTs during the JCP season because TXB₂ is synthesized by them at substantial levels. Changes in the EBC TXB₂ levels, but not the EBC LTB₄ or PGD₂-MOX levels, were more likely correlated with changes in the FEV₁ or %FEV₁. Interestingly, these

Table 3

Correlation between the changes in EBC eicosanoid concentration and changes of pulmonary function or FeNO in patients with asthma with AR.

	CysLTs		LTB ₄		PGD ₂		TXB ₂	
	JCP (+)	JCP (–)	JCP (+)	JCP (–)	JCP (+)	JCP (–)	JCP (+)	JCP (–)
FEV ₁	–0.52*	–0.25	–0.24	0.19	–0.28	0.10	–0.39****	0.25
%FEV ₁	–0.55*	–0.45	–0.27	–0.09	–0.27	0.07	–0.40****	–0.05
FVC	–0.42**	–0.44	–0.18	–0.21	0.03	0.30	–0.18	0.11
%FVC	–0.38	–0.06	–0.27	0.10	–0.13	0.36	–0.15	0.46
FEV ₁ /FVC	–0.53*	0.15	–0.48***	0.41	–0.57*	0.03	–0.52*	0.15
FeNO	0.004	0.34	0.02	0.08	0.06	–0.18	–0.04	0.08

R-values are described. Values of four EBC eicosanoids and FeNO have been log-transformed. Statistically significant values are shown in bold.

* $p < 0.01$; ** $p < 0.04$; *** $p < 0.02$; **** $p = 0.056$; ***** $p < 0.05$.

AR, allergic rhinitis; JCP (+), sensitized to Japanese cedar pollen; JCP (–), not sensitized to Japanese cedar pollen.

increases in the inflammatory eicosanoids were correlated with a decrease in the FEV₁/FVC in the BA-SAR-JCP group (Table 2). A previous study reported that following endobronchial allergen challenge, TBX₂ and PGD₂ were increased in BALF.²⁸ PGD₂ also activates mast cells to produce CysLTs. Consequently, these eicosanoids may interact with CysLTs and each other, thereby exerting supplement modification of pulmonary function.

The present study has several limitations. First, almost all participants in this study were allergic to allergens other than JCP, which may have influenced the seasonal measurement trends owing to possible complementary contributions to allergic inflammation progressing to asthma and AR.³⁸ Second, several patients were treated with ICS and LTRA in this study, indicating that the natural production and effects of eicosanoids might have been reduced. Third, this study included a small number of patients recruited from a single medical institution; thus, some results may be biased. Therefore, further studies using larger cohorts are required to verify these results.

In conclusion, the present study showed that eicosanoid concentrations in the airway increased in the BA-SAR-JCP group during the JCP season, resulting in pulmonary function modification. An increase in CysLT levels may decrease the pulmonary function of patients with asthma sensitized to JCP without clinical symptoms. The findings of this study indicate that a persistent Th2-type mediator levels, such as eicosanoids, are seasonally amplified in patients with asthma with SAR sensitized to specific allergens and that pulmonary function decreases upon exposure to high pollen levels, even in patients using maintenance treatments.

Acknowledgments

The present study was supported by the Japan Society for the Promotion of Science KAKENHI Grant Number JP15K09188 and Grant-in-Aid for Young Physicians from Saitama Medical University Hospital Grant Number 24-C-1-13 and 26-E-1-06.

The authors thank Ms. Akemi Yokote for her excellent technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2020.04.014>.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

TS designed the study, performed the examination, and wrote the manuscript. YU and RH contributed to the collection of data. KN contributed to the interpretation of the results. MN designed the study and interpreted the results. All authors

discussed the results and implications and have read and approved the final manuscript.

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Supplementary Table 1

Comparison of concentration of eicosanoids in EBC during the JCP (on) and non-JCP (off) seasons between the BA-SAR-JCP and BA-AR-non-JCP groups

	JCP-SAR (<i>n</i> = 29)			Non-JCP-AR (<i>n</i> = 13)		
	Off-pollen season	On-pollen season	<i>p</i> -value	Off-pollen season	On-pollen season	<i>p</i> -value
CysLTs,* pg/mL	1.39 (0.63)	1.78 (0.55) ^{†,‡}	0.01	1.41 (0.55)	1.39 (0.38)	n.s.
LTB ₄ ,* pg/mL	0.91 (0.56)	1.14 (0.40)	n.s.	1.03 (0.44)	1.04 (0.33)	n.s.
PGD ₂ ,* pg/mL	0.64 (0.27)	0.72 (0.19)	n.s.	0.78 (0.38)	0.65 (0.16)	n.s.
TXB ₂ ,* pg/mL	0.84 (0.28)	0.92 (0.32) ^{§,}	0.05	0.73 (0.19)	0.65 (0.16)	n.s.

*All values are presented as geometric means (± SD).

JCP-SAR, patients with asthma and seasonal allergic rhinitis sensitized to Japanese cedar pollen; non-JCP-AR, patients with asthma and

allergic rhinitis not sensitized to Japanese cedar pollen.

[†] $p = 0.04$; [§] $p = 0.03$ for JCP-SAR vs. non-JCP-AR during JCP season.

[‡] $p = 0.01$; ^{||} $p = 0.05$ for JCP-SAR during JCP season vs. non-JCP season.