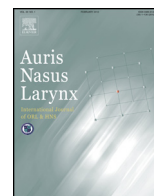




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## Differences in urinary leukotriene E4 levels and distribution of eosinophils between chronic rhinosinusitis patients with aspirin-intolerant and -tolerant asthma

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### ABSTRACT

**Objective:** Urinary leukotriene E4 (U-LTE4) concentrations are significantly elevated in patients with aspirin-intolerant asthma (AIA). However, the relationship between the clinicopathogenetic features of eosinophilic rhinosinusitis and U-LTE4 concentration remains unknown. Here we examined the relationship between U-LTE4 level and eosinophil in chronic rhinosinusitis.

**Methods:** We measured the U-LTE4 concentrations and eosinophil counts in ethmoidal and maxillary sinuses and peripheral blood in 30 asthmatic patients (including 15 AIA patients).

**Results:** Eosinophil counts in ethmoidal sinuses and peripheral blood were markedly higher in asthmatic patients than in controls. Although there were no significant differences between eosinophil counts in maxillary and ethmoidal sinuses for ATA group, eosinophil counts were higher in ethmoidal sinus compared to that in maxillary sinus in the AIA group ( $P < .05$ ). Eosinophil counts were higher in the maxillary than in ethmoidal sinuses for control patients ( $P < .05$ ). Despite low correlation between eosinophil counts in peripheral blood and eosinophil counts in maxillary sinus ( $rs = 0.4323$ ,  $P < .001$ ), moderate correlation was observed between eosinophil counts in peripheral blood and eosinophil counts in ethmoidal sinus ( $rs = 0.5249$ ,  $P < .0001$ ). Basal U-LTE4 concentrations were higher in AIA patients than in those with aspirin-tolerant asthma. Despite low correlation between eosinophil counts and U-LTE4 concentration in maxillary sinus ( $rs = 0.3849$ ,  $P < .01$ ), moderate correlation was observed between eosinophil counts and U-LTE4 concentrations in ethmoidal sinus ( $rs = 0.4736$ ,  $P < .001$ ).

**Conclusion:** We describe the differences in U-LTE4 and other parameters in AIA compared to ATA, and correlation among parameters. We demonstrate that eosinophil-dominant inflammation starts in ethmoidal sinus clinicopathogenetically in CRS with asthma. U-LTE4 concentration was not exclusively associated with eosinophil counts in ethmoidal sinus. Eosinophils in ethmoidal sinus may be a major production site for CysLTs, particularly in AIA. CRS with AIA is assumed to be characterized by leukotriene-eosinophil cross-interaction in ethmoidal sinus.

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## 1. Introduction

Some types of chronic rhinosinusitis (CRS) are less responsive to standard therapy and have a higher tendency to recur. Strong eosinophil infiltration may be observed in the nasal polyps and lamina propria of patients with a type of CRS, known as eosinophilic CRS (ECRS). This term was coined by Haruna et al. in 2001 [1]. ECRS is characterized by inflammation in the ethmoid sinus, anosmia, high frequency of nasal polyp recurrence, and increased eosinophil count in the nasal membrane and blood. However, the study by Haruna et al. described the inflammatory characteristics by computed tomographic evaluation without the evaluation of histological eosinophil distribution.

Steinke et al. reported that eosinophilic nasal polyp tissue contains and produces a large amount of cysteinyl leukotrienes (CysLTs) and that there is a close relationship between CysLT production and eosinophil accumulation in eosinophilic nasal polyps [2].

Nasal polyp tissue may produce CysLTs in case of aspirin-intolerant asthma (AIA) and aspirin-tolerant asthma (ATA), and such CysLTs may have systemic effects. However, Steinke et al. did not describe any quantitative differences in eosinophilic infiltration among the sinuses. We can determine the primary site of inflammation and leukotriene production by anatomically and quantitatively studying eosinophilic infiltration.

CysLTs are important mediators that cause potent bronchoconstriction, mucosal edema, and increased mucus secretion within the airways of asthmatic patients. CysLTs are produced by eosinophils, mast cells, basophils, and monocytes [3,4]. They help impede the cycle in which CysLTs and chemotactic factors are produced by eosinophils via CysLT1 receptors on eosinophils. In contrast, leukotriene E4 (LTE4) has been identified as a major metabolite of LTC4 [5,6], and U-LTE4 is now considered to be the most reliable index for monitoring endogenous CysLT synthesis [7,8].

Therefore, we hypothesized that CysLTs make CRS intractable, and we studied the eosinophilic infiltration of the ethmoid and maxillary sinus mucosa, following extraction during ESS and preoperative CysLTs.

The aims of this study were to determine the (1) characteristics of CRS with bronchial asthma based on anatomical eosinophil distribution and (2) characteristics of CRS with AIA.

## 2. Materials and methods

### 2.1. Subjects

This hospital-based, case–control study was conducted from July 2009 to December 2014. The study comprised 58 patients (mean age, 51.0 years; range, 23–75 years; 21 males and 37 females). CRS diagnosis was based on the European Position Paper on Rhinosinusitis and Nasal Polyps [9]. We divided the patients with CRS into three groups: (1) CRS with AIA, (2) CRS with ATA, and (3) CRS without bronchial asthma (control). There were 15 patients with AIA (mean age, 51.1 years; range, 28–72 years; 4 males and 11 females),

15 with ATA (mean age, 50.6 years; range, 33–73 years; 6 males and 9 females), and 28 non-asthmatic controls who showed bilateral sinusitis on computed tomography (mean age, 51.1 years; range, 23–75 years; 11 males and 17 females; Table 1). Asthma diagnosis was based on the American Thoracic Society criteria [10]. Before and after ESS for CRS, all patients were clinically stable, with no recent changes in asthma symptoms or medications. The patients were receiving only inhaled steroids, leukotriene receptor antagonists, and short-acting  $\beta_2$ -agonists, but no systemic corticosteroids. The diagnosis of AIA was made on the basis of a positive result obtained in an aspirin challenge test or an obvious history of asthma attacks precipitated by NSAID administration. The patients with ATA and healthy controls had a negative aspirin challenge test result or took aspirin or other NSAIDs without any adverse symptoms [11]. The patients who had serum IgE levels of greater than 350 U/mL [11] were classified as atopic. No patient had cystic fibrosis, immotile cilia syndrome, Churg–Strauss syndrome, or autoimmune diseases, and none had upper respiratory tract infections during the six weeks immediately preceding the study. Permission to conduct the study was obtained from the ethics Committee of the National Sagami-hara Hospital, and all patients provided informed consent.

### 2.2. Study design

We collected blood, urine, and sinus tissue samples from the patients. We determined peripheral blood eosinophil counts using a standard automated cell counter. Because  $\beta_2$ -agonists, inhaled corticosteroids, sodium cromoglycate, or oral leukotriene receptor antagonists do not affect U-LTE4 levels [12], these medications were not withheld at the time of urine sample collection. To confirm the relationship between CRS and increased U-LTE4 excretion level, we compared LTE4 urine concentrations within one month before elective endoscopic surgery for CRS.

### 2.3. Clinical evaluation

All enrolled patients subsequently provided a detailed medical history, and they were examined to evaluate the presence of asthma and questioned regarding the use of medications, particularly topical nasal and systemic corticosteroids.

### 2.4. Pathological examination

The mucosal tissues of patients with CRS were removed from the nasal polyps or ethmoid and maxillary sinus at the time of functional ESS. We obtained the maxillary sinus membrane and polyps around membranous portions and the ethmoid sinus mucosa following uncinata process resection. The tissue was immediately fixed in 10% formalin, embedded in paraffin, and cut into thin sections. The sections were stained with hematoxylin–eosin. Nasal polyps were categorized as ethmoid or maxillary tissue with a stem base. We used the proposed diagnosis of Haruna et al. as a reference for eosinophilic sinusitis [1]. The number of eosinophils in the mucosa was counted under high-power field (400 $\times$ ) for fields in which eosinophils were the densest cellular infiltrate beneath the

**Table 1**

Demographic characteristics of the patients with AIA, ATA, and control.

|                                                            | AIA                  | ATA                 | Control             | P value |
|------------------------------------------------------------|----------------------|---------------------|---------------------|---------|
| No.                                                        | 15                   | 15                  | 28                  |         |
| Male/female gender                                         | 4/11                 | 6/9                 | 11/17               | NS      |
| Age (y), mean (SD)                                         | 51.1 ( $\pm 14.5$ )  | 50.6 ( $\pm 13.3$ ) | 51.1 ( $\pm 14.6$ ) | NS      |
| Blood eosinophil percentage (%), median (range)            | 8.7 (2.2–25.3)       | 7.6 (2.2–25.3)      | 4.1 (0.1–10.9)      | *       |
| Atopic: serum IgE levels $\geq 350$ U/mL No.               | 0                    | 8                   | 7                   |         |
| U-LTE4 (pg/mg creatinine), median (range)                  | 437.6 (163.8–4322.7) | 205.9 (51.2–742.1)  | 148.5 (71.4–268.4)  | **      |
| Eosinophil counts in maxillary sinus mucosa median (range) | 79.7 (3–163.7)       | 20.7 (0.7–174)      | 8.5 (0–79.3)        | ***     |
| Eosinophil counts in ethmoid sinus mucosa median (range)   | 106.7 (18.7–207.3)   | 26.3 (0.7–201.3)    | 5.8 (0–40.7)        | ****    |
| Systemic corticosteroids                                   | 3                    | 2                   | 2                   |         |
| Topical nasal corticosteroids                              | 10                   | 9                   | 6                   |         |
| Inhaled corticosteroids                                    | 12                   | 13                  | 3                   |         |
| Oral leukotriene receptor antagonists                      | 11                   | 7                   | 4                   |         |

NS, not significant.

\* There was a significant difference between CRS with bronchial asthma (including AIA and ATA) compared to control ( $P < .01$ ), but no significant difference between AIA and ATA.\*\* There was a significantly higher difference in the AIA compared to control ( $P < .0001$ ) and ATA ( $P < .01$ ), but no significant difference between ATA and control.\*\*\* There was a significant difference between AIA and control ( $P < .0001$ ), AIA and ATA ( $P < .05$ ), but no significant difference between ATA and control.\*\*\*\* There was a significantly higher difference in the AIA compared to control ( $P < .0001$ ), and AIA to ATA group ( $P < .01$ ), and between ATA and control ( $P < .01$ ).

epithelial surface. Histological examinations were performed, and the mean number of eosinophils was calculated.

### 2.5. Quantification of CysLTs in urine

We measured LTE4 concentrations in spot urine as previously described [11,13–15]. The U-LTE4 concentration was expressed as picograms per milligram of creatinine.

### 2.6. Data analysis

Demographic characteristics are expressed as mean  $\pm$  standard deviation. The U-LTE4 concentration, ethmoid and maxillary sinus eosinophil counts, serum IgE levels, and peripheral blood eosinophil percentage were reported as medians and ranges. The initial analysis was between CRS patients with and without asthma, followed by the subanalysis between ATA and AIA using the Wilcoxon rank-sum test. We compared eosinophil distribution between the ethmoid and maxillary sinuses using the paired-Wilcoxon test for the AIA, ATA, and control groups. Relationships were analyzed using the Spearman rank correlation test between U-LTE4 concentrations and eosinophil counts in the ethmoid sinus mucosa, maxillary sinus mucosa, and peripheral whole blood.  $P$ -values of  $< .05$  were considered to be statistically significant.

## 3. Results

Table 1 shows the clinical characteristics of patients in the AIA, ATA, and control groups. These three groups were well matched by age and gender. U-LTE4 concentrations did not differ with gender. Table 1 shows the atopic, systemic, topical nasal, and inhaled corticosteroids.

### 3.1. Pathology

Eosinophil counts in the ethmoid sinus mucosa were significantly higher in CRS with bronchial asthma (including

AIA and ATA) compared with those in the controls ( $P < .001$ ). Although there were no significant differences between eosinophil counts in the maxillary and ethmoid sinus mucosa for the ATA group, eosinophil counts were higher in the ethmoid sinus than in the maxillary sinus in the AIA group ( $P < .05$ , Fig. 1). In addition, eosinophil counts were higher in the maxillary sinus than in the ethmoid sinus in the controls ( $P < .05$ , Fig. 1).

### 3.2. Blood eosinophils

Peripheral blood eosinophil percentages were significantly higher in CRS with bronchial asthma (including AIA and ATA) compared with those in the controls ( $P < .01$ ; Table 1). Although no significant differences between the AIA and ATA groups with respect to peripheral blood eosinophil percentages

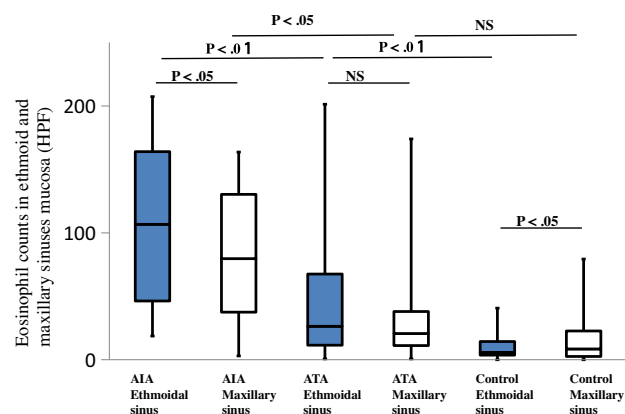


**Fig. 1.** Eosinophil counts in ethmoid and maxillary sinuses mucosa (high-power field, HPF; 400 $\times$ ), expressed as medians plus percentiles (3rd quartile + 1.5 interquartile range, 25%, 75%, and 1st quartile – 1.5 interquartile range). The first analysis should be between CRS patients with and without asthma and then the sub-analysis between ATA and AIA by using Wilcoxon rank sum test.

were observed, the ATA group had a wider distribution of peripheral blood eosinophils.

### 3.3. U-LTE4 expression

U-LTE4 concentrations were significantly higher in the AIA group (median 437.6, range 163.8–4322.7 pg/mg creatinine) than in the control group (median 148.5, range 71.4–268.4 pg/mg creatinine) and ATA group (median 205.9, range 51.2–742.1 pg/mg creatinine; Fig. 2).

### 3.4. Correlation analysis of parameters

There was a moderate correlation between eosinophil counts in the ethmoid sinus mucosa and U-LTE4 concentrations ( $r_s = 0.4736$ ,  $P < .001$ ; Fig. 3A).

There was low correlation between eosinophil counts in the maxillary sinus and total U-LTE4 concentrations ( $r_s = 0.3849$ ,  $P < .01$ ; Fig. 3B). However, there was no significant correlation between peripheral blood eosinophil percentages and U-LTE4 concentrations ( $r_s = 0.2274$ , NS).

A moderate correlation between eosinophil counts in the ethmoid sinus mucosa and peripheral blood eosinophil percentages ( $r_s = 0.5249$ ,  $P < .0001$ ; Fig. 3C) was observed. A moderate correlation between eosinophil counts in the maxillary sinus mucosa and peripheral blood eosinophil counts ( $r_s = 0.4323$ ,  $P < .001$ ; Fig. 3D) was observed.

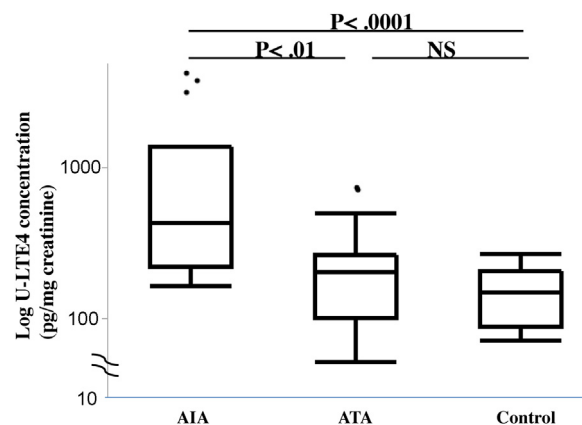


**Fig. 2.** U-LTE4 concentration (in picograms per milligram of creatinine) in the AIA, ATA, and control groups. U-LTE4 concentrations are expressed as medians plus percentiles (3rd quartile + 1.5 interquartile range, 25%, 75%, and 1st quartile – 1.5 interquartile range). The first analysis should be between CRS patients with and without asthma and then the sub-analysis between ATA and AIA by using Wilcoxon rank sum test.

## 4. Discussion

CRS without bronchial asthma is associated with less sinus tissue eosinophilia and lack of a specific anatomical site, whereas CRS with asthma is characterized by eosinophil-dominant inflammation with distribution in both the ethmoid

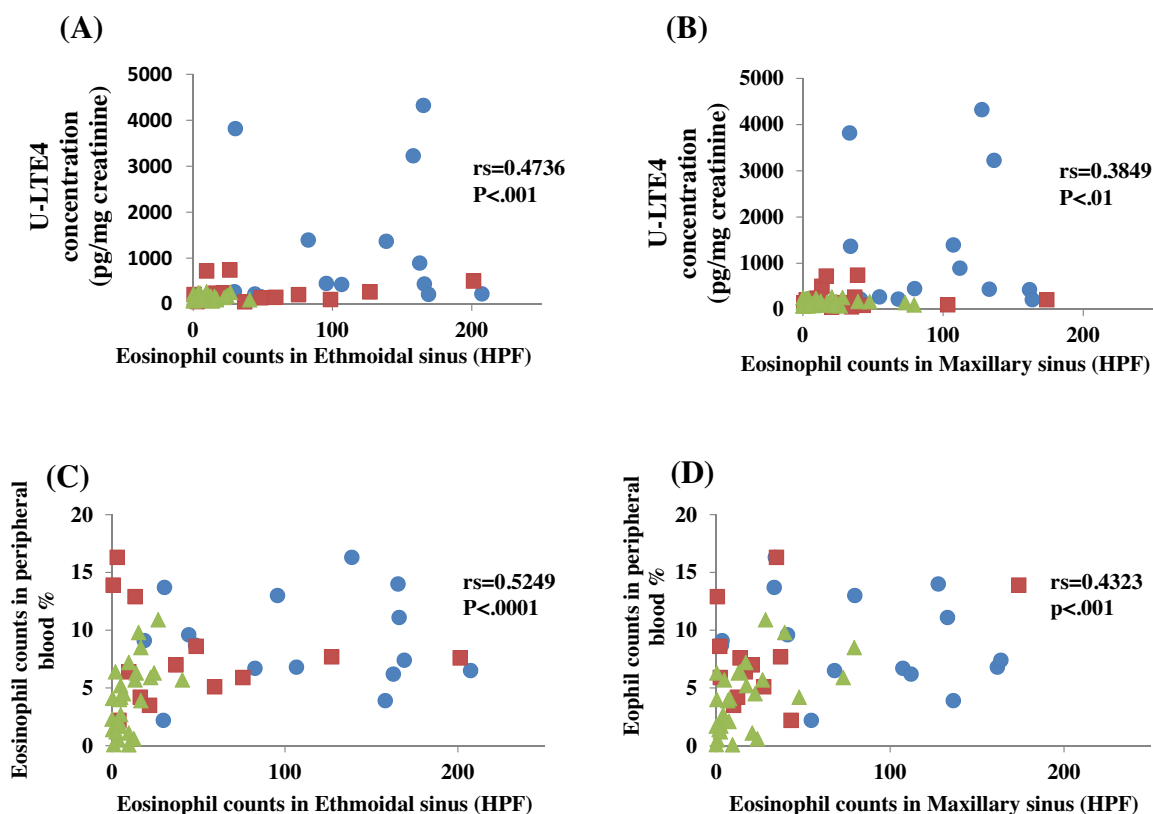


**Fig. 3.** AIA, ATA, and control groups are denoted by a circle, a square and a triangle, respectively. Correlation analysis was performed using Spearman's rank correlation coefficient. (A) Correlation between the eosinophil counts in ethmoid sinus mucosa (high-power field, HPF, 400 $\times$ ) and U-LTE4 concentration (pg/mg creatinine). (B) Correlation between the eosinophil counts in maxillary sinus mucosa (high-power field, HPF; 400 $\times$ ) and U-LTE4 concentration (pg/mg creatinine). (C) Correlation between the eosinophil counts in ethmoid sinus mucosa (HPF, 400 $\times$ ) and peripheral blood eosinophil counts ( $\times 10^6$ /mL). (D) Correlation between the eosinophil counts in maxillary sinus mucosa (HPF; 400 $\times$ ) and peripheral blood eosinophil counts ( $\times 10^6$ /mL).

and maxillary sinuses. Furthermore, CRS with AIA is assumed to be characterized by leukotriene–eosinophil cross-interaction in the ethmoid sinus. We demonstrated that eosinophil-dominant inflammation clinicopathogenetically starts in the ethmoid sinus in cases of CRS with asthma. Eosinophils in the ethmoid sinus may be a major production site for CysLTs, particularly in AIA.

We could not elucidate the reason for the difference in eosinophil counts between the ethmoid and maxillary sinuses. AIA could occur due to acquired sensitivity and result in increased U-LTE4 production.

On comparing the ethmoid to the maxillary sinus, the ethmoid cellular structure may increase blood flow, thereby increasing the correlation between the ethmoid sinus and peripheral blood eosinophil counts. It is believed that most cases of intractable CRS are associated with non-atopic bronchial asthma such as AIA. Such cases are characterized by the same indicators as asthma that relate to the clinical conditions of CRS [16].

The precise pathophysiological mechanism underlying hyperleukotrienuria remains unclear. Increased U-LTE4 concentrations have been observed not only in ECRS but also in autoimmune diseases including rheumatoid arthritis [17] and systemic lupus erythematosus [18].

Considering the high recurrence rate of nasal polyposis in patients with AIA [19], we should examine whether those with CRS recurrence have significantly increased U-LTE4 levels over time and whether aspirin intolerance develops in patients with ATA and controls with hyperleukotrienuria who exhibit clinical characteristics similar to those exhibited by patients with AIA. We should examine CysLTs in the ethmoid and maxillary sinuses to clarify pathological conditions with respect to local eosinophilic inflammation.

The ESS resection of large nasal polyps and sinonasal mucosa normalization help impede the cycle through which CysLTs and chemotactic factors are produced in the nasal sinus tissue [20] to promote eosinophil chemotaxis [21]. In addition, ESS improves drug delivery systems through the nose to the maxillary sinus.

In conclusion, we demonstrated, for the first time, important clinicopathogenetic features and that the eosinophil distribution in the ethmoid and maxillary sinuses is associated with increased U-LTE4 secretion levels in CRS.

## Conflict of interest

None of the authors has any conflict of interest, financial, or otherwise.

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