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## Original Article

## Implication of fraction of exhaled nitric oxide and blood eosinophil count in severe asthma

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## List of Abbreviation:

b-EOS, blood eosinophil count;  
CAP, Capsulated hydrophilic carrier  
polymer; GINA, Global Initiative for asthma;  
IQR, interquartile range; high FeNO/high  
b-EOS, FeNO  $\geq 25$  and b-EOS count  $\geq 300$ ;  
high FeNO/low b-EOS, FeNO  $\geq 25$  and b-EOS  
count  $< 300$ ; low FeNO/high b-EOS, FeNO  
 $< 25$  and b-EOS count  $\geq 300$ ; low FeNO/low  
b-EOS, FeNO  $< 25$  and b-EOS count  $< 300$ ;  
OCS, Oral corticosteroids; ppb, Part per  
billion

## ABSTRACT

**Background:** Severe asthma is a complex disease with heterogeneous features and involves type 2 airway inflammation, including eosinophil accumulation. Surrogate biomarkers, fraction of exhaled nitric oxide (FeNO) and blood eosinophil count (b-EOS), may predict eosinophilic airway inflammation. Here we investigated clinical characteristics of severe asthma phenotype using a combined analysis of FeNO and b-EOS.

**Methods:** This retrospective study examined clinical data of patients with severe asthma (N = 107; median age, 64 years) treated at Saitama Medical University Hospital from 2009 to 2016. Thresholds of FeNO and b-EOS for sputum eosinophil ratio  $\geq 2\%$  were determined using receiver operating characteristic curve (ROC) analysis. Clinical characteristics were analyzed after classifying patients into four subgroups according to these thresholds.

**Results:** Of 39 induced sputum samples examined, ROC area under the curve for predicting sputum eosinophilia was 82.0% (p = 0.001) for b-EOS and 77.0% (p = 0.006) for FeNO at optimal cut-off values of  $\geq 300/\mu\text{L}$  and  $\geq 25$  ppb, respectively. The number of sensitized allergens was higher in the high FeNO/low b-EOS and high FeNO/high b-EOS subgroups (p < 0.05). The prevalence of chronic sinusitis was higher in the low FeNO/high b-EOS and high FeNO/high b-EOS subgroups (p = 0.04). The high FeNO/high b-EOS subgroup included the largest proportion (approximately 40%) of patients experiencing frequent severe exacerbations. Both low FeNO/low b-EOS and high FeNO/low b-EOS subgroups showed less severe exacerbations.

**Conclusions:** Combined evaluation of FeNO and b-EOS can identify patients with frequent exacerbations and stratify the appropriate therapy for type 2 inflammation-predominant severe asthma.

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## Introduction

Severe asthma is well known to be a heterogeneous disease that comprises multiple factors, such as genetics, immune cascades, physiologic indices, and environmental triggers. Moreover, the presence of severe asthma predisposes approximately 10% of patients to suffer daily symptoms and acute exacerbations despite the intake of high-dose inhaled corticosteroids, oral corticosteroids (OCS), and other controllers.<sup>1,2</sup> To improve therapeutic precision,

evidence on the connection between the phenotype and endotype of severe asthma has emerged.<sup>2–7</sup> Recently, identification of severe asthma mediated by type 2 inflammation has resulted in the successful launch of several biological therapeutics that target such inflammation, especially primary eosinophilic inflammation.<sup>8–13</sup>

Monitoring the number of eosinophils in both airway and blood is beneficial and feasible in the management of asthma.<sup>8–15</sup> Therapeutic strategy based on the airway eosinophil number comprises the improvement of daily condition and exacerbation risk in mild-to-severe asthma, while if it is based on the blood eosinophil number,<sup>14,15</sup> then it primarily comprises the prevention of exacerbations in only severe asthma.<sup>8–13,16</sup> Biological therapeutics reduced the odds ratios of exacerbation risk in severe asthmatic patients with blood eosinophil number  $\geq 150$ –500 cells/ $\mu\text{L}$  unless

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their airway eosinophil numbers are within the normal level.<sup>8–10,16,17</sup> Actually, in mild-to-severe asthma, patients with airway and systemic eosinophilia have more severe asthma status but those with only airway eosinophilia show the deterioration of pulmonary function, compared with those without eosinophilia.<sup>18</sup> Thus, classification into four categories of severe asthmatics on the basis of the combination of airway and blood eosinophilia could allow us to strictly identify eosinophilic asthma.

The management plan is according to eosinophilia in the sputum or airway eosinophilia, which is defined as a sputum eosinophil ratio that conventionally ranges from 2% to 3%.<sup>5,14,15</sup> Nevertheless, analyzing the sputum for inflammatory cellular profiles in general clinics may be suboptimal because it is slightly invasive and entails some amount of time. Recently, fraction of exhaled nitric oxide (FeNO) and blood eosinophil (b-EOS) count have been reported as useful predictors of eosinophilia in the sputum referred as sputum eosinophilia.<sup>17,19–22</sup> However, studies on FeNO and b-EOS count reported diverse thresholds for determining airway eosinophilia.<sup>17,19–22</sup> To the best of our knowledge, there have been no studies that have determined the cut-off values of FeNO and b-EOS count in Japan. Validation of the cut-off values to identify airway eosinophilia could help establish the characteristics of severe asthma with underlying predominant type 2 inflammation.

Several biomarkers have been reported to characterize patients with severe asthma with predominantly type 2 inflammation.<sup>7,17,23–27</sup> FeNO is an endogenous inflammatory product that is primarily delivered from activated bronchial epithelium in response to type 2 cytokines, such as IL-13; in other words, increases in FeNO reflect type 2 inflammation that is induced by Th2 immune promoters, as well as the number of eosinophils in the airway.<sup>28</sup> Likewise, increases in b-EOS count have been reported to be a likely initiator of progression of allergic airway inflammation, a potential indicator of generalized type 2 inflammation, and one of the factors independently associated with chronic sinusitis.<sup>29,30</sup> Increased levels of FeNO and b-EOS count, surrogate biomarkers of sputum eosinophilia, were independent risk factors for severe asthma exacerbation,<sup>16,31–36</sup> and the combined use of these two type 2 inflammatory biomarkers markedly increased the positive odds ratio for severe asthma exacerbation.<sup>36</sup> Therefore, we hypothesized that in the real clinical field, FeNO and b-EOS count could individually serve as pivotal biomarkers of localized and generalized type 2 inflammation in severe asthma. The potential of these biomarkers to strongly identify a severe type 2 inflammation-predominant asthma characterized by eosinophilia can lead to personalized treatment of such severe asthma cases.

This study was conducted to determine the profile of severe asthma with type 2 allergic inflammation using two methods of measuring eosinophilic inflammation. Because the reported standard values for b-EOS count and FeNO in measuring airway eosinophilia vary, we aimed to determine the cut-off values for these markers in outpatients with severe asthma. Subsequently, we clarified the features of type 2 inflammation-predominant severe asthma by dividing the patients into four subgroups based on b-EOS count and FeNO, which represented generalized and localized eosinophilic inflammation, respectively.

## Methods

### Study design and subjects

We conducted a retrospective cohort study to determine the characteristics of patients with type 2 inflammation-predominant severe asthma (107 outpatients, age 20–84 years) who were seen at the Allergy Center and the Department of Respiratory Medicine

of Saitama Medical University Hospital from 2009 to 2016. The data were basically collected from the electronic medical records of outpatients. To determine the optimum cut-off values of b-EOS count and FeNO to identify a sputum eosinophil count of  $\geq 2\%$ , the data of patients who had successful sputum induction were collected from the other ongoing clinical studies in our hospital (approval numbers: 08–043 and 13–083). The study was approved by the institutional review board of the Saitama Medical University Hospital (approval number: 16–046). Written informed consent was obtained from all individuals.

The inclusion criterion was a physician diagnosis of severe asthma based on the Japanese Society of Allergy guideline,<sup>37</sup> which corresponded with treatment action steps 4–5 of the Global Initiative for Asthma Guidelines.<sup>38</sup> The patients had a clear clinical history of asthma symptoms and showed  $\geq 12\%$  reversibility of the predicted value of FEV1 and/or airway hyperresponsiveness (PC20 methacholine  $< 8$  mg/mL). The exclusion criteria included the presence of a lung disease other than asthma that can interfere with the evaluation; asthma exacerbation for at least 4 weeks before the day of outpatient consult; respiratory infections within the preceding month of the day of outpatient consult; pregnancy; comorbid diseases, including cancer, severe renal failure, or severe heart failure; and current or more than 10 pack-year smoking history.

We assessed the clinical presentation in terms of demographics, duration of asthma, number of sensitized aeroallergens, allergic comorbidity, asthma medications, asthma exacerbations, pulmonary function, b-EOS count, serum IgE, FeNO collected from the medical records, and sputum and eosinophil ratio. Using capsulated hydrophilic carrier polymer (CAP), the specific IgE antibodies of the following common aeroallergens in Japan were measured: house dust mites, Japanese cedar pollen, Japanese cypress, ragweed, orchard grass, candida species, alternaria, aspergillus species, dog, and cat. A positive sensitized inhalant allergen was designated as  $> 0.35$  UA/mL by CAP. We confirmed that the patients diagnosed as having chronic sinusitis were examined by an otolaryngologist or underwent computed tomography.

The number of asthma exacerbations was counted for 12 months after the date of b-EOS count and FeNO measurement. Based on the American Thoracic Society (ATS)/European Respiratory Society statement, asthma exacerbation was defined as mild to moderate, when the deterioration in symptoms and/or lung function with increased rescue bronchodilator use lasted for 2 days or more, and as severe, when there was a need for hospitalization or two or more courses of OCS.<sup>39</sup> Pulmonary function tests were performed using an AS307 spirometer (Minato Medical Science, Osaka, Japan).<sup>40</sup> FeNO was measured using a chemiluminescence analyzer (SiEVER 280i, Nippon Megacare, Tokyo, Japan) with a resolution of 1 part per billion (ppb), according to the recommendations of the ATS.<sup>41</sup>

### Sputum induction and processing

Subjects performed sputum induction, as described previously.<sup>42,43</sup> Briefly, after premedication with salbutamol using a metered-dose inhaler, sterile hypertonic saline (4.5%) was inhaled at room temperature through an ultrasonic nebulizer (MU-32, Azwell, Osaka, Japan). Thereafter, sputum was induced at 5-min intervals for up to 20 min.

The collected sputum samples were immediately processed, prepared on cytospin slides (Cytospin 3; Shandon, Tokyo, Japan), which were stained with May–Grunwald–Giemsa. An independent investigator counted at least 500 inflammatory cells; the presence of  $< 50\%$  squamous epithelial cells in the samples was considered as adequate for analysis.<sup>44</sup>

## Statistical analysis

The normality of the values was determined using the Kolmogorov–Smirnov test or the Leven test. Comparison of two groups used the Student *t*-test for parametric continuous variables and the Mann–Whitney U-test for nonparametric variables. Comparison of data among three or more groups used analysis of variance for parametric data and the Kruskal–Wallis test for nonparametric data. Associations were determined by the Spearman correlation test. Categorical variables were tested by the  $\chi^2$  test or the Fisher exact test, as appropriate. Values were described as mean  $\pm$  standard deviation (SD) or as median with 25th–75th percentile range, if not normally distributed.

In the beginning of the study, we evaluated the diagnostic accuracy of FeNO and b-EOS count in identifying the optimal cut-off value for sputum eosinophils  $\geq 2\%$  with the use of receiver operating characteristic curve (ROC) analysis for each individual variable. An ROC area under the curve (AUC) of 0.5 was designated as an inability of the biomarker to predict a subject's correct classification. We adopted the clinically relevant cut-off values for 2% sputum eosinophils from previous studies.<sup>5,44,45</sup> Based on the derived cut-off values, the sensitivity, specificity, positive predictive value, and negative predictive value were calculated.

Subsequently, using the optimal cut-off values of FeNO (in ppb) and b-EOS count (in cells/ $\mu$ L), we divided the eligible patients into the following four groups:

- 1) FeNO <25 and b-EOS count <300 (low FeNO/low b-EOS);
- 2) FeNO  $\geq 25$  and b-EOS count <300 (high FeNO/low b-EOS);
- 3) FeNO <25 and b-EOS count  $\geq 300$  (low FeNO/high b-EOS); and
- 4) FeNO  $\geq 25$  and b-EOS count  $\geq 300$  (high FeNO/high b-EOS).

When we compared the proportions of patients who experienced severe or mild to severe acute exacerbations, the number of severe exacerbations was divided into three categories: none, 1 or 2, and  $\geq 3$  and the number of mild to severe exacerbations was divided into three categories: none or 1, 2–5, and  $\geq 6$ . The data were analyzed using SPSS version 20.0 (IBM, Armonk, New York, USA).

## Results

A total of 128 severe asthmatic patients were selected from the electronic medical records, including 56 patients who performed sputum induction. Of patients undergoing sputum induction, 14 were excluded because of mild or moderate exacerbation and 6 because of  $\geq 50\%$  squamous epithelial cells in the sputum samples. One patient was excluded because of no data regarding FeNO. Lastly, 107 patients who met the inclusion criteria and had no exacerbation in previous 4 weeks at the day of beginning the study were examined. Of all patients, 5 had slight cough or dyspnea, but they did not require additional medications in the previous 4 weeks. The demographic and clinical characteristics of the study population are shown in Table 1. The median age was 64 years [interquartile range (IQR), 51–73 years]. Of all patients, 78 (72.9%) had concomitant allergic disease and 55 (51.4%) were confirmed to have chronic sinusitis. Most patients were treated with high-dose inhaled corticosteroids (ICS) and long-acting  $\beta$ -agonist or leukotriene receptor antagonist, whereas 30% and 10% of all patients were treated with OCS and omalizumab, respectively. There were 36 patients (33.6%) in whom sputum induction was successful; the squamous cell ratio in these samples was 12.2% (IQR, 4.3%–42.1%). Compared with the entire study population of patients with severe asthma, the patients who provided adequate sputum measurements had similar age, smoking history, number of sensitized allergens, intake of medications, total IgE, b-EOS count, and FeNO, but

**Table 1**

Demographic and clinical characteristics of the subjects.

|                                         | All patients                   | Patients who underwent sputum induction |
|-----------------------------------------|--------------------------------|-----------------------------------------|
| N (%)                                   | 107                            | 36 (33.6%)                              |
| Age, y                                  | 64.0 (51.0–73.0)               | 63.5 (46.5–71.8)                        |
| Female/male, n                          | 63/44                          | 13/23                                   |
| BMI, kg/m <sup>2</sup>                  | 23.3 (6.1–24.8)                | 24.0 (21.5–26.8)                        |
| Smoking history, no/past/present, n (%) | 58 (54.2)/42 (39.3)/7 (6.5)    | 17 (47.2)/16 (44.4)/3 (8.3)             |
| Duration of asthma (y)                  | 13.5 (4.0–21.0)                | 5.6 (2.1–12.3)                          |
| Sensitized allergen, n                  | 2.0 (0.0–4.0)                  | 3.0 (1–4.8)                             |
| Allergic rhinitis, n (%)                | 67 (62.6)                      | 23 (63.9)                               |
| Chronic sinusitis, n (%)                | 34 (31.8)                      | 12 (33.3)                               |
| Atopic dermatitis, n (%)                | 19 (17.8)                      | 9 (25.0)                                |
| Urticaria, n (%)                        | 19 (17.8)                      | 4 (11.1)                                |
| ICS dose ( $\mu$ g/d)                   | 1000 (800–1200)                | 800 (800–1000)                          |
| OCS dose (mg/d)                         | 0 (0.0–3.0)                    | 0 (0.0–0.0)                             |
| LABA/LT/Theophylline, n (%)             | 100 (93.5)/96 (89.7)/52 (48.6) | 35 (97.2)/31 (86.1)/28 (77.8)           |
| Oral corticosteroids, n (%)             | 32 (29.9)                      | 8 (22.2)                                |
| Anti-IgE antibody, n (%)                | 10 (9.3)                       | 8 (22.2)                                |
| Total Log IgE, IU/L                     | 2.3 (0.6)                      | 2.5 (0.6)                               |
| Blood eosinophil count,/ $\mu$ L        | 270 (119–559)                  | 365 (190–678)                           |
| FeNO, ppb                               | 21.0 (11.0–38.0)               | 19.5 (11.3–38.0)                        |
| Sputum eosinophil ratio, %              | NA                             | 2.2 (0.3–16.4)                          |
| FEV <sub>1</sub> , % predicted          | 85.8 (23.2)                    | 71.3 (21.9)                             |
| FVC, % predicted                        | 94.5 (22.5)                    | 83.6 (16.4)                             |
| FEV <sub>1</sub> /FVC, %                | 67.9 (14.8)                    | 68.3 (14.5)                             |

Parametric data are expressed as mean (SD); nonparametric data are expressed as median (25%–75%). ICS = inhaled corticosteroid (fluticasone); 2  $\mu$ g beclomethasone = 2  $\mu$ g budesonide = 1  $\mu$ g fluticasone. LABA, long-acting  $\beta$ -agonist; LT, leukotriene receptor antagonist.

had shorter duration of asthma, higher prevalence of omalizumab intake, and lower %predicted FEV<sub>1</sub>.

## The optimal cut-off values of b-EOS count and FeNO for sputum eosinophilia

The sputum eosinophil ratio was correlated with the b-EOS count and FeNO (Supplementary Fig. 1a, b). The ROC curve analysis showed that the diagnostic accuracies of a b-EOS count of  $\geq 300$  cells/ $\mu$ L and an FeNO level  $\geq 25$  ppb to distinguish between eosinophilic and noneosinophilic airway inflammation were 82.0% [ $p = 0.001$ , 95% confidence interval (CI): 67.3–95.8] and 77.0% [ $p = 0.006$ , 95% CI: 67.3–92.6], respectively (Fig. 1), with 75.0%

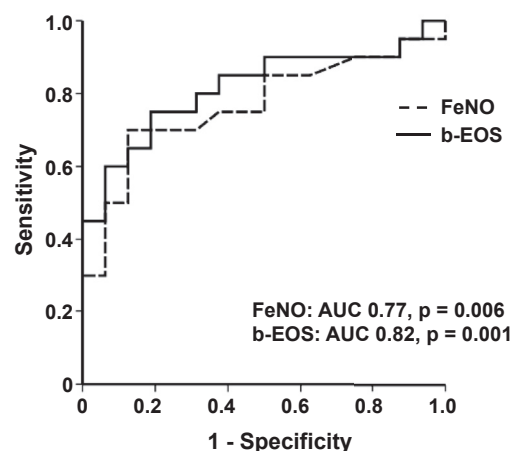


**Fig. 1.** Receiver-operating characteristic curve analysis for individual blood eosinophil count and FeNO levels to differentiate between sputum eosinophil <2% and sputum eosinophil  $\geq 2\%$ . The AUCs are similar for the two parameters of eosinophilia (Table 2). AUC, area under the curve.

sensitivity and 68.8% specificity and 65.0% sensitivity and 87.5% specificity, respectively (Table 2). Because the diagnostic performance of these parameters was comparable with the previously reported evidence, we decided to use these cut-off values in the entire population.

#### Clinical characteristics of the four subgroups

Using the b-EOS count and the FeNO cut-off values, all patients with severe asthma were stratified into the following four subgroups: 39 in the low FeNO/low b-EOS subgroup, 17 in the high FeNO/low b-EOS subgroup, 23 in the low FeNO/high b-EOS subgroup, and 28 in the high FeNO/high b-EOS subgroup (Table 3). The prevalence rates of severe eosinophilic asthma based on the b-EOS count ( $n = 51$ , 47.7%) and the FeNO ( $n = 45$ , 42.1%) were comparable. There were no differences among the groups in terms of sex, age, body mass index, duration of asthma, and prevalence of allergic comorbidities. The prevalence of

chronic sinusitis was higher in the high b-EOS subgroups than in the low b-EOS subgroups (Table 3). The median number of sensitized to common aeroallergens was higher in the high FeNO/low b-EOS subgroup (4.5, IQR: 0.0–7.5) than in the low FeNO/low b-EOS (1.0, IQR: 1.0–4.0); the low FeNO/high b-EOS (1.0, IQR: 0.0–3.0); and the high FeNO/high b-EOS (3.0, IQR: 1.0–4.8) subgroups ( $p = 0.035$ , Fig. 2). Among the four subgroups, the total number of acute exacerbations was the highest in the high FeNO/high b-EOS subgroup, but there were no differences in the ICS dose and in the proportion of patients taking OCS, omalizumab, and additional asthma controller medications. In all subgroups, the mean pulmonary function test results, except for the FEV<sub>1</sub>/FVC ratio, were normal. Of the patients who were able to provide adequate sputum samples, 13 patients (36.1%) had sputum eosinophil ratio of  $\geq 2\%$ : 3 (23.1%) in the low FeNO/low b-EOS, 3 (23.1%) in the high FeNO/low b-EOS, 1 (7.7%) in the low FeNO/high b-EOS, and 6 (46.2%) in the high FeNO/high b-EOS subgroups (Table 3).

**Table 2**  
Summary of the ROC curve analyses to predict sputum eosinophil percentages  $<2\%$  and  $\geq 2\%$ .

|                        | Cut-off value | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) | Accuracy (%) |
|------------------------|---------------|-----------------|-----------------|-------------------------------|-------------------------------|--------------|
| FeNO (ppb)             | 22.5          | 70.0            | 81.2            | 82.4                          | 68.4                          | 75.0         |
|                        | 25            | 65.0            | 87.5            | 86.7                          | 66.7                          | 75.0         |
|                        | 28.5          | 60.0            | 87.5            | 85.7                          | 63.6                          | 72.2         |
| b-EOS (cells/ $\mu$ L) | 271           | 80.0            | 68.7            | 76.2                          | 73.3                          | 75.0         |
|                        | 300           | 75.0            | 68.8            | 75.0                          | 68.8                          | 72.2         |
|                        | 330           | 75.0            | 75.0            | 78.9                          | 70.6                          | 75.0         |

b-EOS, blood eosinophil counts.

**Table 3**  
Demographic, functional, and inflammatory characteristics according to the predominant type 2 inflammation phenotype.

|                                     | Low FeNO, Low b-EOS           | High FeNO, Low b-EOS           | Low FeNO, High b-EOS             | High FeNO, High b-EOS            | p value among the groups |
|-------------------------------------|-------------------------------|--------------------------------|----------------------------------|----------------------------------|--------------------------|
| N (%)                               | 39 (36.4)                     | 17 (15.9)                      | 23 (21.5)                        | 28 (26.2)                        |                          |
| Age, y                              | 63.0 (45.0–72.0)              | 66.0 (59.5–77.0)               | 70.0 (47.0–76.0)                 | 62.0 (56.3–69.3)                 | N.S.                     |
| Female/male, n                      | 24/15                         | 8/9                            | 14/9                             | 17/11                            | N.S.                     |
| BMI, kg/m <sup>2</sup>              | 23.5 (4.2)                    | 23.5 (4.3)                     | 22.5 (20.4–25.4)                 | 24.9 (5.6)                       | N.S.                     |
| Smoking history, no/past/present, n | 21/17/1                       | 9/5/3                          | 10/12/1                          | 18/8/2                           | N.S.                     |
| Duration of asthma (y)              | 16.1 (13.3)                   | 21.5 (13.3)                    | 14.5 (10.0)                      | 13.9 (12.5)                      | N.S.                     |
| Sensitized allergen, n              | 1.0 (1.0–4.0)                 | <b>4.5 (0.0–7.5)*</b>          | 1.0 (0.0–3.0)                    | <b>3.0 (1.0–4.8)**</b>           | <b>0.035</b>             |
| Allergic rhinitis, n (%)            | 25 (64.1)                     | 11 (64.7)                      | 13 (56.5)                        | 18 (64.3)                        | N.S.                     |
| Chronic sinusitis, n (%)            | 7 (17.9)                      | 4 (23.5)                       | <b>11 (47.8)***</b>              | <b>12 (42.9)****</b>             | <b>0.04</b>              |
| Atopic dermatitis, n (%)            | 7 (17.9)                      | 1 (5.9)                        | 6 (26.1)                         | 5 (17.9)                         | N.S.                     |
| Urticaria, n (%)                    | 7 (17.9)                      | 3 (17.6)                       | 5 (21.7)                         | 4 (14.3)                         | N.S.                     |
| ICS dose ( $\mu$ g/d)               | 1000 (800–1200)               | 1000 (800–1300)                | 800 (800–1200)                   | 1000 (925–1400)                  | N.S.                     |
| OCS dose (mg/d)                     | 0 (0.0–3.0)                   | 0 (0.0–0.0)                    | 0 (0.0–5.0)                      | 0 (0.0–1.0)                      | N.S.                     |
| LABA/LT/TP, n (%)                   | 37 (94.9)/37 (94.9)/21 (53.8) | 16 (94.1)/16 (94.1)/4 (23.5)   | 21 (91.3)/21 (91.3)/14 (60.9)    | 26 (92.9)/22 (78.6)/13 (46.4)    | N.S.                     |
| OCS, n (%)                          | 11 (28.2)                     | 2 (11.8)                       | 11 (47.8)                        | 8 (28.6)                         | N.S.                     |
| Mild to moderate AE                 | 0.0 (0.0–1.0)                 | 0.0 (0.0–1.0)                  | 0.0 (0.0–2.0)                    | 1.0 (0.0–2.0)                    | N.S.                     |
| Severe AE                           | 1.0 (0.0–2.0)                 | 0.0 (0.0–1.5)                  | 1.0 (0.0–2.0)                    | 1.5 (0.0–4.0)                    | N.S.                     |
| Total of AE                         | 1.0 (0.0–1.0)                 | 1.0 (0.0–1.0)                  | 2.0 (3.0–4.0)                    | <b>3.0 (1.0–6.0)****, ****</b>   | <b>0.04</b>              |
| Anti-IgE antibody, n (%)            | 3 (7.7)                       | 2 (11.8)                       | 3 (13.0)                         | 2 (7.1)                          | N.S.                     |
| Total Log IgE, IU/L                 | 2.2 (0.5)                     | 2.5 (0.9)                      | 2.3 (0.7)                        | 2.5 (0.5)                        | N.S.                     |
| Blood eosinophil/ $\mu$ L           | 129 (60–213)                  | 139 (56–229)                   | <b>543 (355–667)*****, *****</b> | <b>598 (392–852)*****, *****</b> | <b>&lt;0.0001</b>        |
| FeNO, ppb                           | 12.6 (6.1)                    | <b>43.3 (18.9)*****, *****</b> | 15.6 (5.6)                       | <b>61.7 (33.5)*****, *****</b>   | <b>&lt;0.0001</b>        |
| Sputum Eos ratio <sup>†</sup> , %   | 0.2 (0.0–1.5)                 | 2.0 (1.8–19.2)                 | 7.8 (0.4–12.2)                   | <b>17.6 (3.4–39.6)*****</b>      | <b>0.005</b>             |
| Sputum Neu ratio <sup>†</sup> , %   | 23.0 (8.7–67.2)               | 27.6 (14.5–58.3)               | 41.8 (15.7–66.6)                 | 40.6 (28.8–56.8)                 | N.S.                     |
| FEV <sub>1</sub> , % predicted      | 87.5 (24.8)                   | 96.0 (22.3)                    | 80.1 (18.0)                      | 82.3 (24.0)                      | N.S.                     |
| FVC, % predicted                    | 97.0 (23.1)                   | 106.2 (20.5)                   | 85.6 (20.8)                      | 91.6 (21.8)                      | N.S.                     |
| FEV <sub>1</sub> /FVC, %            | 69.8 (16.7)                   | 65.1 (15.5)                    | 67.8 (13.7)                      | 67.0 (12.7)                      | N.S.                     |

Significant p values are shown in boldface. Parametric data are expressed as mean (SD); nonparametric data are expressed as median (25%–75%). ANOVA and the Kruskal–Wallis test were performed for parametric continuous variables and nonparametric variables. Categorical variables were tested by the  $\chi^2$  test or Fisher exact test, as appropriate. Further exploration of the results with significant differences in the initial analyses was performed by *post hoc* pairwise analyses with Bonferroni correction or by the Mann–Whitney U-test, as appropriate.

ICS = inhaled corticosteroid (fluticasone); 2  $\mu$ g beclomethasone = 2  $\mu$ g budesonide = 1  $\mu$ g fluticasone.

\* $p = 0.044$ , \*\* $p = 0.02$ , \*\*\*\*\* $p < 0.0001$ ; vs low FeNO, high b-EOS.

\*\*\* $p = 0.01$ , \*\*\*\* $p = 0.03$ , \*\*\*\*\* $p < 0.0001$ , \*\*\*\*\* $p = 0.007$ ; vs low FeNO, low b-EOS; \*\*\*\*\* $p = 0.02$ ; \*\*\*\*\* $p < 0.0001$ ; vs high FeNO, low b-EOS.

b-EOS, blood eosinophils; LABA, long-acting  $\beta_2$ -agonist; LT, leukotriene receptor antagonist; AE, acute exacerbation; OCS, oral corticosteroids; Eos, eosinophil; Neu, neutrophil.

<sup>†</sup> Number, n (%): All asthmatic subjects 36; low FeNO, low b-EOS 12 (33.3); low FeNO, high b-EOS 9 (25.0), high FeNO, low b-EOS 4 (11.1), high FeNO, high b-EOS 11 (30.6).

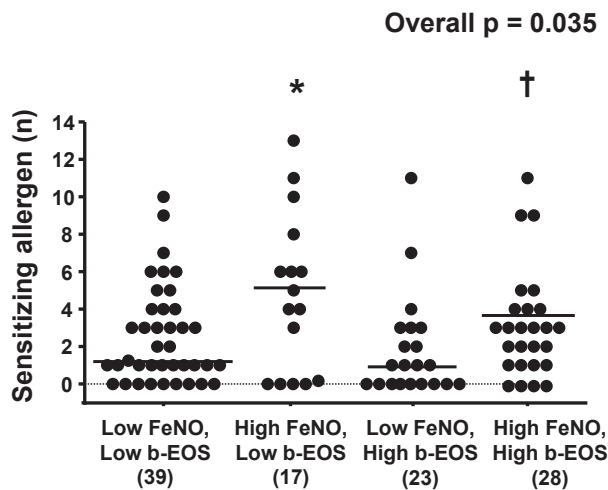


**Fig. 2.** Differences in the number of sensitized aeroallergens among the predominant type 2 inflammation phenotypes. Horizontal bars represent median. Overall  $p = 0.035$ . \*vs low FeNO, low b-EOS,  $p = 0.044$ ; †vs low FeNO, high b-EOS,  $p = 0.02$ . b-EOS, blood eosinophils.

Patients with high FeNO/high b-EOS accounted for more than 25% of the study population, the second largest proportion among the four subgroups. Over 40% of these patients had chronic sinusitis; this was nearly the same as the proportion of patients with low FeNO/high b-EOS and was significantly larger than that of patients with low FeNO/low b-EOS (Table 3). Those who with high FeNO/high b-EOS were sensitized to a significantly larger number of common aeroallergens, compared with that in patients with low FeNO/high b-EOS (vs 1.0, IQR: 0.0–3.0,  $p = 0.02$ , Fig. 2). Approximately 40% of patients with high FeNO/high b-EOS experienced  $\geq 3$  severe asthma exacerbations and approximately 30% experienced  $\geq 6$  mild to severe asthma exacerbations; both proportions were significantly higher than those in patients with low b-EOS (Fig. 3a, b).

Patients with low FeNO/low b-EOS accounted for approximately 40% of all patients, showing the largest population among the four subgroups. These patients accounted for the smallest proportion of patients with concomitant chronic sinusitis ( $p = 0.04$ ). Moreover, those patients showed a tendency to have less number of allergens to which they were sensitized, compared with that in patients with high FeNO/low b-EOS subgroup ( $p = 0.06$ ). The total number of exacerbations was significantly less in this subgroup than in the high b-EOS subgroups, especially comparing the high FeNO/high b-EOS subgroup ( $p = 0.03$ ). Less than 20% of those with low FeNO/low b-EOS had  $\geq 3$  severe asthma exacerbations and only less than 10% of them had  $\geq 6$  mild to severe asthma exacerbations. Those proportions were significantly lower than the corresponding proportions in the high FeNO/high b-EOS subgroup (Fig. 3a, b).

Patients with low FeNO/high b-EOS comprised one-fifth of all patients. Approximately 50% of these patients, which is the highest proportion among the subgroups, had concomitant chronic sinusitis. Those patients had a significantly less number of common aeroallergens to which they were sensitized, compared with that in patients with high FeNO/low b-EOS and with high FeNO/high b-EOS (Fig. 2). Patients with low FeNO/high b-EOS exhibited the second-largest proportion of patients who had  $\geq 3$  severe asthma exacerbations among the four subgroups (overall  $p = 0.03$ , Fig. 3a). The proportion of patients having  $\geq 6$  mild to severe asthma exacerbations was small in this subgroup and was comparable with that in the low b-EOS subgroups (overall  $p = 0.015$ , Fig. 3b).

Patients with high FeNO/low b-EOS accounted for the smallest proportion (less than 20%) of all patients. This subgroup had a

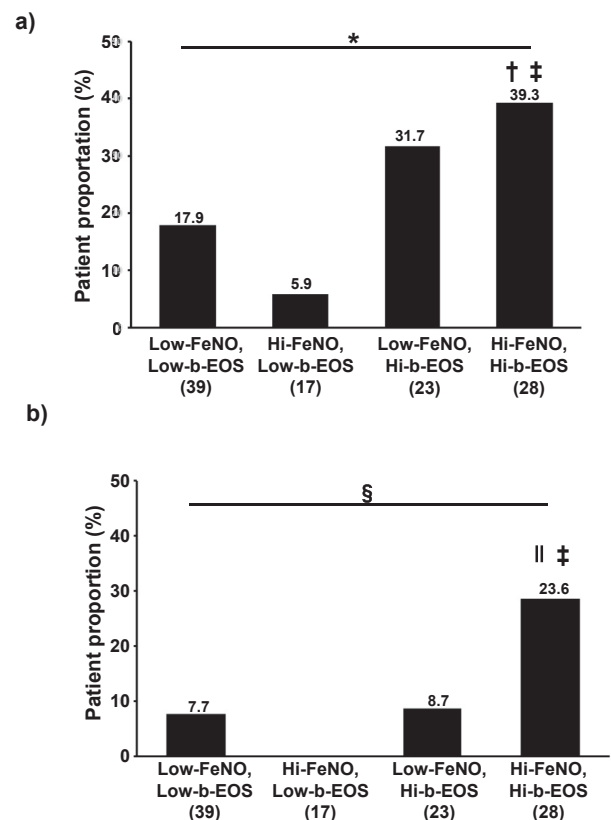


**Fig. 3.** Distribution of patients with frequent severe and mild to severe asthma exacerbations in the predominant type 2 inflammation phenotypes. **a)** Proportion of patients with  $\geq 3$  episodes of severe asthma exacerbation. **b)** Proportion of patients with  $\geq 6$  episodes of asthma exacerbation of all severities. The  $\chi^2$  test and Fisher exact test were calculated for comparison of proportions. \* $p = 0.03$ , † $p = 0.015$ , overall. ‡ $p < 0.05$ , § $p = 0.03$ , vs low FeNO, low b-EOS, † $p = 0.014$ , vs high FeNO, low b-EOS. b-EOS, blood eosinophils; AE, asthma exacerbation.

modest but higher proportion (nearly 25%) of patients with chronic sinusitis, compared with patients with low FeNO/low b-EOS (overall  $p = 0.015$ , Table 3). Those patients had a significantly higher number of common aeroallergens to which they were sensitized, compared with that in patients with low FeNO/high b-EOS (Fig. 2). Those patients had a tendency to have better %predicted FEV<sub>1</sub> and %predicted FVC, compared with the values in the other subgroups. The smallest proportion of these patients had  $\geq 3$  severe asthma exacerbations, which was significant when compared with that in patients with high FeNO/high b-EOS (Fig. 3a). There was no patient with  $\geq 6$  mild to severe asthma exacerbations (Fig. 3b).

The proportion of patients having severe asthma exacerbations of different frequencies varied among the subgroups (Fig. 4a). In the high FeNO/high b-EOS subgroup, this proportion gradually increased up to approximately 40% at  $\geq 3$  severe exacerbations along with the increasing category of severe exacerbation numbers. On the contrary, in the low FeNO/low b-EOS and the high FeNO/low b-EOS subgroups, nearly half of the patients had no severe exacerbations; the prevalence of patients with severe exacerbations gradually decreased with the increasing number of exacerbations. Especially, in the high FeNO/low b-EOS subgroup, the proportion of patients with 1 or 2 severe asthma exacerbations was more than 40% and was higher than that in the low FeNO/low b-EOS subgroup. In the low FeNO/high b-EOS subgroup, 30% had no exacerbation and 10% had 1 or 2 severe exacerbations. But in the high FeNO/high b-EOS subgroup, the proportion of patients with  $\geq 3$  severe exacerbations increased to 30%.

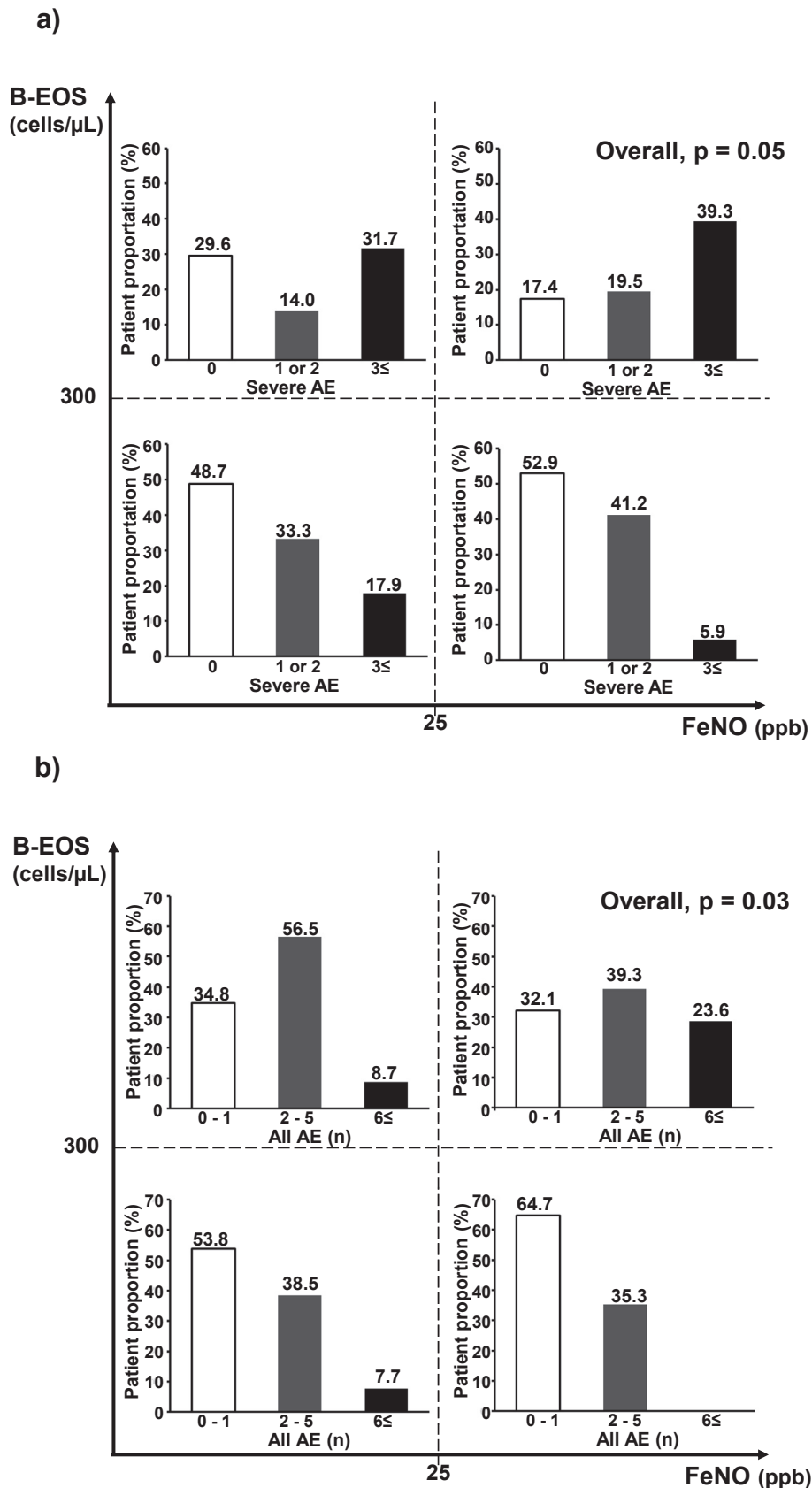


**Fig. 4.** Distribution of patients with severe and mild-to-severe asthma exacerbations in the predominant type 2 inflammation phenotypes. **a)** Proportion of patients according to the frequency of severe asthma exacerbation and classified by the FeNO and b-EOS cut-off points for sputum eosinophilia. **b)** Proportion of patients according to the frequency of asthma exacerbation of all severities and classified by the FeNO and b-EOS cut-off values for sputum eosinophilia. The  $\chi^2$  test and Fisher exact test statistics were calculated for comparison of proportions. b-EOS, blood eosinophil; AE, asthma exacerbation.

The proportion of patients having mild to severe asthma exacerbations of different frequencies varied among the subgroups (Fig. 4b). In the high FeNO/high b-EOS subgroup, the proportions of patients in the three categories of mild to severe asthma exacerbation frequency were comparable. In the two subgroups of patients with low b-EOS, those proportions of patients having mild to severe asthma exacerbations decreased along with the increasing category of mild to severe exacerbation numbers. In the two subgroups of patients with high b-EOS, the proportion of patients having  $\geq 2$  exacerbations was higher than that in the two subgroups of patients with low b-EOS. The proportion of patients having 2–5 exacerbations of any severity was the largest (approximately 60%) in the low FeNO/high b-EOS subgroup.

## Discussion

This study that classified patients with severe asthma into four subtypes according to b-EOS count and FeNO cut-off values to identify eosinophilia in the sputum disclosed several clinical characteristics of severe asthma with predominant type 2 inflammation. The prevalence of patients with severe asthma having frequent acute severe exacerbation, chronic sinusitis, and number of sensitized aeroallergens was different among the four subgroups. In particular, the prevalence of frequent exacerbations was the largest in severe asthmatic subjects with high FeNO and high b-EOS and gradually decreased as the b-EOS count and FeNO level decreased; these findings were similar to those previously reported.<sup>31–33,35,36</sup> Notably, this present study focused on adult patients with severe asthma, whereas the previous studies included patients with mild to severe asthma and/or corticosteroid-naïve asthma in all age groups. This discrepancy suggested that patients with severe asthma exhibit heterogeneity in type 2 inflammation and that measurement of the b-EOS count and FeNO in such patients may help in the formulation of a therapeutic strategy and comprehensive achievement of asthma control. Our results might be valuable when considering targets for biological therapeutics in clinical practice.

Since the high FeNO and high b-EOS are likely recognized as the airways and potentiate systemic type 2 inflammation, respectively, the patients with high FeNO/high b-EOS can be typically interpreted as having authentic severe eosinophilic asthma in this study. These patients experienced highly frequent severe exacerbations, the highest number of overall exacerbation, and had a high prevalence of chronic sinusitis. These characteristics of severe asthma were in agreement with the findings of several studies, which showed the independent association of higher FeNO, b-EOS, and/or serum-eosinophil cationic protein with acute severe exacerbation; the involvement of both high sputum eosinophil ratio and high b-EOS count with unstable asthma; and the capability of a high b-EOS count to predict chronic sinusitis.<sup>30,36,46,47</sup> Therefore, a widespread type 2 inflammation is likely to hasten the progression of poorly controlled severe asthma. Additionally, approximately one-third of those patients had persistent local and systemic type 2 inflammation that was resistant to OCS and high-dose ICS (Table 3). Therefore, severe asthmatic patients classified in the high FeNO/high b-EOS subgroup may be the ideal phenotype that could benefit from novel type 2 therapies, such as the anti-IL-5 antibody.

By contrast, severe asthmatic subjects with low FeNO and low b-EOS, the largest proportion among the four subgroups, exhibited no specific characteristics, except for the relatively less frequent severe exacerbations. The features in this subgroup were similar to those in asthmatic subjects with paucigranulocytes in the sputum who exhibited mildly decreased pulmonary function, moderate use of OCS, and modest rate of acute exacerbation.<sup>5,48</sup> On the contrary, more than one-third of those with low FeNO/low b-EOS

experienced frequent severe asthma exacerbations and moderate rate of exacerbation of any severity in this study (Figs. 3 and 4). Actually, in this study, 23% of patients with eosinophilia in the sputum belonged to the low FeNO/low b-EOS subgroup. Therefore, the patients with low FeNO/low b-EOS in this study might exhibit complex features of paucigranulocytic and eosinophil-predominant asthma.

Patients with low FeNO and high b-EOS accounted for only 20% of all patients. The asthma status in these patients seemed to markedly progress to a poorly controlled condition, based on the results that these patients accounted for the largest proportion of patients having moderately frequent acute exacerbations (Fig. 4b) and 30% of those with  $\geq 3$  severe exacerbations. These observations seem to verify that a high b-EOS appears as a risk factor for severe exacerbation.<sup>31–33,36</sup> The b-EOS shows higher association with sputum eosinophilia than with FeNO in asthma.<sup>20,21</sup> In the present study, a b-EOS count of  $>300$  cells/ $\mu$ L had good sensitivity and modestly good negative and positive predictive values for sputum eosinophilia. One study on repeated sputum induction demonstrated that 20% of patients with mild-to-moderate asthma treated with ICS intermittently increased the sputum eosinophils ratio,<sup>49</sup> suggesting that in severe asthmatic subjects, eosinophilia in the sputum may intermittently occur even though the FeNO value was low. In fact, mean sputum eosinophil ratio was approximately 8% in this study (Table 3). Therefore, patients with low FeNO/high b-EOS may potentially be under a prodromal stage of airway eosinophilia and be suitable targets for anti-IL-5 therapy because b-EOS is continuously driven by IL-5.<sup>29</sup>

Severe asthmatic subjects with high FeNO alone presented with interesting characteristics, including sensitization to more environmental allergens that could trigger the upregulation of the Th2 immune cascade. FeNO is a highly reactive gaseous mediator through conversion of *L*-arginine to *L*-citrulline by inducible nitric oxide synthase (iNOS), which is enhanced and released by IL-13 in bronchial epithelial cells.<sup>34</sup> Following allergen provocation, the NOS activity and expression of iNOS were increased in the bronchial epithelium of asthmatic subjects with high FeNO.<sup>50</sup> Other studies showed that the seasonal exposure to or the persistently exposure to the higher level of sensitizing aeroallergens increased the odds ratio for seasonal severe asthma exacerbation or number of hospitalizations, respectively.<sup>51,52</sup> Taken together, localized type 2 inflammation in the airway is probably mediated by localized IL-4/IL-13 mechanisms that are triggered by inhalant allergens<sup>53</sup> and seems to selectively drive a high FeNO-predominant type of severe asthma, leading to occasional poorly controlled asthma and/or moderate number of exacerbations. In this study, the majority in this subtype have moderate frequency of exacerbation, which is similar with the result in previous studies.<sup>36</sup> In addition, a recent study demonstrated that higher FeNO ( $\geq 35$  ppb) exhibited good prediction for high CCL26 gene expression in airway mucosa which is the most highly expressed selective IL-13-driven gene,<sup>54</sup> which suggests that high FeNO acts as a biomarker for identifying patients with IL-13-promoted inflammation, particular type 2 inflammation, in airway. These IL-4/IL-13-promoted mechanisms might be the targets for treatment with biological agents, such as anti-IL-4 receptor or anti-IgE.

In this study, the sensitivity and specificity of FeNO  $\geq 25$  ppb and b-EOS  $\geq 300$  cells/ $\mu$ L in predicting airway eosinophilia were similar to the results of some studies, showing the AUC 0.83 for b-EOS and 0.82 for FeNO, approximately 70–80% sensitivity and approximately 70–90% specificity at a b-EOS count of 220/ $\mu$ L or at 270/ $\mu$ L, approximately 60–75% sensitivity and specificity at a FeNO level of 20 ppb or 24 ppb and in mild to severe asthma.<sup>20,31,49</sup> One meta-analysis showed that the sensitivity and specificity in detecting  $\geq 3\%$  sputum eosinophilia were 66% and 76%, respectively, for FeNO

and 71% and 77%, respectively, for blood eosinophils.<sup>26</sup> Our results confirmed better accuracies for identifying sputum eosinophil ratio  $\geq 2\%$  than those reported in one study, which showed accuracy rates of 63% for FeNO  $\geq 30$  ppb and 66% for b-EOS  $\geq 300$  cells/ $\mu$ L in mild to severe asthma.<sup>45</sup> Therefore, the cut-off values of FeNO and b-EOS for sputum eosinophilia in this study were likely feasible.

There are several parameters to categorize severe asthma; these include inflammatory cells in sputum or blood, cytotoxic proteins, mediators, and genetic variations.<sup>5–7,24–27</sup> Although measurements of type 1 or type 2 inflammation, or Th1 or Th2 immune system, are popular indexes, these classifications do not demonstrate the characteristics of the distinct endotypes of severe asthma; rather, they reflect complex airway inflammation that comprise eosinophil and neutrophil infiltration of varying degrees according to the disease severity.<sup>5</sup> In this study, we classified severe asthma into four categories using authentic cut-off values that reflect the influence of type 2 inflammation. This simple approach successfully emphasized the patients with type 2 inflammation-predominant severe asthma that might be suitable for anti-IgE, anti-IL-5, and anti-IL-4 receptor therapies.

There were several limitations in this study. The retrospective design, small sample number, and the single-institution setting might have caused patient selection bias and low validation. The fact that more than 30% of all patients underwent sputum induction might have added some potential biases; patients with undergoing sputum induction exhibited more severe asthma than the whole patients because they had more severe pulmonary function. Therefore, further studies on this issue are needed.

In conclusion, this study demonstrated that the four categories of severe asthma based on the b-EOS count and FeNO, which are surrogate biomarkers of type 2 inflammation, enabled characterization of eosinophilic inflammation and reflected the clinical manifestations of the comorbidities, allergic status, and future risks. These biomarkers are easy to measure in clinical practice and can potentially identify patients with type 2 inflammation-predominant severe asthma who might benefit from biological therapeutics.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.alit.2018.04.003>.

### Conflict of interest

The authors have no conflict of interest to declare.

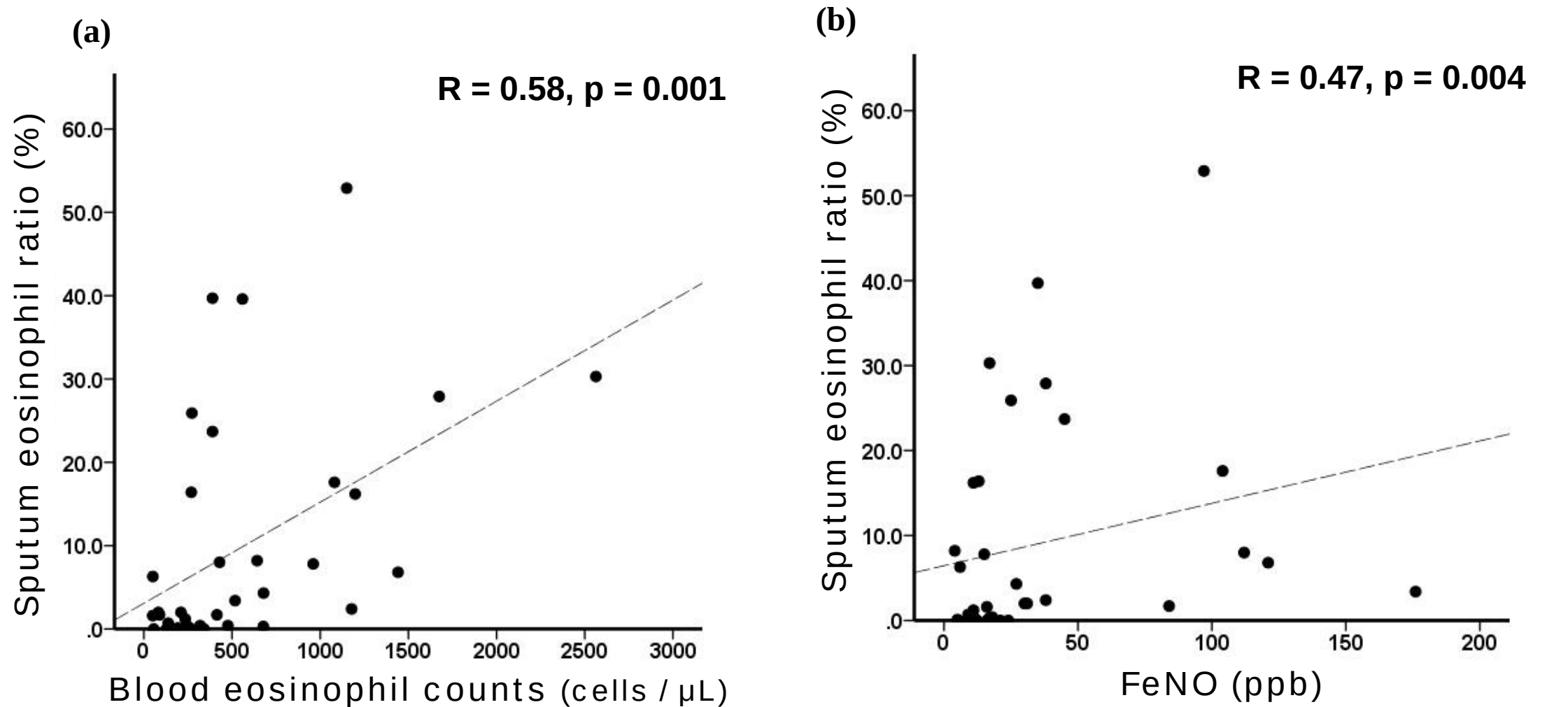
### Authors' contributions

TS designed the study, performed the examination and wrote the manuscript. HI, EN, SM, and YU 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.

## References

- Israel E, Reddel HK. Severe and difficult-to-treat asthma in adults. *N Engl J Med* 2017;**377**:965–76.
- Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;**43**:343–73.
- Hastie AT, Moore WC, Meyers DA, Vestal PL, Li H, Peters SP, et al. National Heart, Lung, and Blood Institute Severe Asthma Research Program. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. *J Allergy Clin Immunol* 2010;**125**:1028–36.
- Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;**18**:716–25.
- Moore WC, Hastie AT, Li X, Li H, Busse WW, Jarjour NN, et al. National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. *J Allergy Clin Immunol* 2014;**133**:1557–63.
- Yan X, Chu JH, Gomez J, Koenigs M, Holm C, He X, et al. Noninvasive analysis of the sputum transcriptome discriminates clinical phenotypes of asthma. *Am J Respir Crit Care Med* 2015;**191**:1116–25.
- Leflaudeux D, De Meulder B, Loza MJ, Peffer N, Rowe A, Baribaud F, et al. U-BIOPRED clinical adult asthma clusters linked to a subset of sputum omics. *J Allergy Clin Immunol* 2017;**139**:1797–807.
- Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 2014;**371**:1198–207.
- Castro M, Zangrilli J, Wechsler ME, Bateman ED, Brusselle GG, Bardin P, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir Med* 2015;**3**:355–66.
- Bleecker ER, FitzGerald JM, Chanez P, Papi A, Weinstein SF, Barker P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dose inhaled corticosteroids and long-acting  $\beta_2$ -agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. *Lancet* 2016;**388**:2115–27.
- Wenzel S, Castro M, Corren J, Maspero J, Wang L, Zhang B, et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting  $\beta_2$  agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. *Lancet* 2016;**388**:31–44.
- Normansell R, Walker S, Milan S, Walters E, Nair P. Omalizumab for asthma in adults and children. *Cochrane Database Syst Rev* 2014;**1**:CD003559.
- Humbert M, Busse W, Hanania NA, Lowe PJ, Canvin J, Erpenbeck VJ, et al. Omalizumab in asthma: an update on recent developments. *J Allergy Clin Immunol Pract* 2014;**2**:525–36. e1.
- Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Pavord ID, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;**360**:1715–21.
- Petsky HL, Li A, Chang AB. Tailored interventions based on sputum eosinophils versus clinical symptoms for asthma in children and adults. *Cochrane Database Syst Rev* 2017;**8**:CD005603.
- Yancey SW, Keene ON, Albers FC, Ortega H, Bates S, Pavord I, et al. Biomarkers for severe eosinophilic asthma. *J Allergy Clin Immunol* 2017;**140**:1509–18.
- Katz LE, Gleich GJ, Hartley BF, Yancey SW, Ortega HG. Blood eosinophil count is a useful biomarker to identify patients with severe eosinophilic asthma. *Ann Am Thorac Soc* 2014;**11**:531–6.
- Schleich FN, Chevremont A, Paulus V, Henket M, Manise M, Louis R, et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. *Eur Respir J* 2014;**44**:97–108.
- Schleich FN, Seidel L, Sele J, Manise M, Quaedvlieg V, Louis R, et al. Exhaled nitric oxide thresholds associated with a sputum eosinophil count  $\geq 3\%$  in a cohort of unselected patients with asthma. *Thorax* 2010;**65**:1039–44.
- Wagner AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJ, Sterk PJ, et al. External validation of blood eosinophils, Fe(NO) and serum periostin as surrogates for sputum eosinophils in asthma. *Thorax* 2015;**70**:115–20.
- Fowler SJ, Tavernier G, Niven R. High blood eosinophil counts predict sputum eosinophilia in patients with severe asthma. *J Allergy Clin Immunol* 2015;**135**:822–4. e2.
- Korevaar DA, Westerhof GA, Wang J, Cohen JF, Spijker R, Bossuyt PM, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis. *Lancet Respir Med* 2015;**3**:290–300.
- Hanania NA, Wenzel S, Rosen K, Hsieh HJ, Mosesova S, Choy DF, et al. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med* 2013;**187**:804–11.
- Busse W, Spector S, Rosen K, Wang Y, Alpan O. High eosinophil count: a potential biomarker for assessing successful omalizumab treatment effects. *J Allergy Clin Immunol* 2013;**132**:485–6. e11.
- Zhang XY, Simpson JL, Powell H, Yang IA, Upham JW, Gibson PG. Full blood count parameters for the detection of asthma inflammatory phenotypes. *Clin Exp Allergy* 2014;**44**:1137–45.
- Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin Immunol* 2014;**133**:388–94.
- Nagasaki T, Matsumoto H, Izuhara K. KiHAC Respiratory Medicine Group. Utility of serum periostin in combination with exhaled nitric oxide in the management of asthma. *Allergol Int* 2017;**66**:404–10.
- Chibana K, Trudeau JB, Mustovich AT, Hu H, Zhao J, Wenzel SE, et al. IL-13 induced increases in nitrite levels are primarily driven by increases in inducible nitric oxide synthase as compared with effects on arginases in human primary bronchial epithelial cells. *Clin Exp Allergy* 2008;**38**:936–46.
- Salter BM, Sehmi R. Hematopoietic processes in eosinophilic asthma. *Chest* 2017;**152**:410–6.
- Asano T, Kanemitsu Y, Takemura M, Yokota M, Fukumitsu K, Niimi A, et al. Serum periostin as a biomarker for comorbid chronic rhinosinusitis in patients with asthma. *Ann Am Thorac Soc* 2017;**14**:667–75.
- Zeiger RS, Schatz M, Li Q, Chen W, Khatri DB, Tran TN, et al. High blood eosinophil count is a risk factor for future asthma exacerbations in adult persistent asthma. *J Allergy Clin Immunol Pract* 2014;**2**:741–50.
- Price DB, Rigazio A, Campbell JD, Bleecker ER, Corrigan CJ, Gopalan G, et al. Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. *Lancet Respir Me* 2015;**3**:849–58.

33. Denlinger LC, Phillips BR, Ramratnam S, Ross K, Bhakta NR, Jarjour NN, et al. National heart, lung, and blood institute's severe asthma research program-3 investigators. Inflammatory and comorbid features of patients with severe asthma and frequent exacerbations. *Am J Respir Crit Care Med* 2017;**195**: 302–13.
34. Dweik RA, Sorkness RL, Wenzel S, Hammel J, Curran-Everett D, Erzurum SC, et al. National Heart, Lung, and Blood Institute Severe Asthma Research Program. Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. *Am J Respir Crit Care Med* 2010;**181**: 1033–41.
35. Petsky HL, Kew KM, Turner C, Chang AB. Exhaled nitric oxide levels to guide treatment for adults with asthma. *Cochrane Database Syst Rev* 2016;**9**: CD011440.
36. Malinovschi A, Fonseca JA, Jacinto T, Alving K, Janson C. Exhaled nitric oxide levels and blood eosinophil counts independently associate with wheeze and asthma events in National Health and Nutrition Examination Survey subject. *J Allergy Clin Immunol* 2013;**132**:821–7. e1-5.
37. Ohta K, Yamaguchi M, Akiyama K, Adachi M, Ichinose M, Takahashi K, et al. Japanese guideline for adult asthma. *Allergol Int* 2011;**60**:115–45.
38. 2015 Global Initiative for Asthma (GINA) report. Global Strategy for Asthma Management and Prevention. Updated 2015. Available at: <http://www.ginasthma.org>.
39. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Wenzel SE, et al. American Thoracic Society/European Respiratory Society Task Force on Asthma Control and Exacerbations. An official American thoracic society/European respiratory society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009;**180**:59–99.
40. Wanger J. Standardization of spirometry, ATS/ERS task force. *Eur Respir J* 2005;**26**:319–38.
41. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;**171**:912–30.
42. Paggiaro PL, Chanez P, Holz O, Ind PW, Djukanovic R, Maestrelli P, et al. Sputum induction. *Eur Respir J* 2002;**37**:3s–8.
43. Fahy JV, Boushey HA, Lazarus SC, Mauger EA, Cherniack RM, Chinchilli VM, et al. Safety and reproducibility of sputum induction in asthmatic subjects in a multicenter study. *Am J Respir Crit Care Med* 2001;**163**:1470–5.
44. Takaku Y, Soma T, Uchida Y, Kobayashi T, Nakagome K, Nagata M. CXC chemokine superfamily induced by Interferon- $\gamma$  in asthma: a cross-sectional observational study. *Asthma Res Pract* 2016;**2**:6.
45. Hastie AT, Moore WC, Li H, Rector BM, Ortega VE, Bleecker ER, et al. National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. *J Allergy Clin Immunol* 2013;**132**:72–80.
46. Mogensen I, Alving K, Bjerg A, Borres MP, Hedlin G, Malinovschi A, et al. Simultaneously elevated exhaled nitric oxide and serum-eosinophil cationic protein relate to recent asthma events in asthmatics in a cross-sectional population-based study. *Clin Exp Allergy* 2016;**46**:1540–8.
47. Malinovschi A, Janson C, Borres M, Alving K. Simultaneously increased fraction of exhaled nitric oxide levels and blood eosinophil counts relate to increased asthma morbidity. *J Allergy Clin Immunol* 2016;**138**:1301–8. e2.
48. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006;**11**: 54–61.
49. McGrath KW, Icitovic N, Boushey HA, Lazarus SC, Sutherland ER, Fahy JV, et al. Asthma clinical research network of the national heart, lung, and blood institute. A large subgroup of mild-to-moderate asthma is persistently non-eosinophilic. *Am J Respir Crit Care Med* 2012;**185**:612–9.
50. Roos AB, Mori M, Grönneberg R, Österlund C, Claesson HE, Nord M, et al. Elevated exhaled nitric oxide in allergen-provoked asthma is associated with airway epithelial iNOS. *PLoS One* 2014;**9**:e90018.
51. Canova C, Heinrich J, Anto JM, Leynaert B, Smith M, Jarvis D, et al. The influence of sensitisation to pollens and moulds on seasonal variations in asthma attacks. *Eur Respir J* 2013;**42**:935–45.
52. Tham R, Vicendese D, Dharmage SC, Hyndman RJ, Newbigin E, Erbas B, et al. Associations between outdoor fungal spores and childhood and adolescent asthma hospitalizations. *J Allergy Clin Immunol* 2017;**139**:1140–7. e4.
53. Alving K, Malinovschi A. Basic aspects of exhaled nitric oxide. In: Horvath I, de Jongste JC, editors. *Exhaled Biomarkers, European Respiratory Society Monograph*. Lausanne: European Respiratory Society; 2010. p. 1–31.
54. Silkoff PE, Laviolette M, Singh D, FitzGerald JM, Kelsen S, Loza MJ, et al. Airways Disease Endotyping for Personalized Therapeutics (ADEPT) study investigators. Identification of airway mucosal type 2 inflammation by using clinical biomarkers in asthmatic patients. *J Allergy Clin Immunol* 2017;**140**: 710–9.



**Supplementary Figure 1. Correlation of sputum eosinophil ratio with blood eosinophil counts and FeNO**

Sputum eosinophil ratio is correlated with (a) blood eosinophil counts and (b) FeNO (N = 36). Associations among them are calculated using the Spearman correlation test.