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Association of upper and lower airway eosinophilic inflammation with response to omalizumab in patients with severe asthma

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ABSTRACT

Background: The anti-immunoglobulin E monoclonal antibody, omalizumab, is used to treat severe asthma and has the potential to ameliorate airway inflammation. However, the effect of omalizumab in ameliorating upper airway inflammation has not been fully elucidated. **Objective:** We investigated the association of upper and lower airway inflammation with the response to omalizumab treatment. **Methods:** We used the Global Evaluation of Treatment Effectiveness to assess the efficacy of omalizumab in treating 16 patients with severe asthma. We also investigated the symptom score, short-acting β -agonist inhaler use, pulmonary function, biomarkers, computed tomography scans, and nasal mucosa pathology at omalizumab initiation and after four months of treatment. **Results:** When the fraction of exhaled nitric oxide (FeNO) and the percentage of sputum eosinophil were used as indicators of lower airway inflammation, positive correlations were found between CD20 B-cell, mast cell, and eosinophil counts in the nasal mucosa. Improved asthma symptoms were observed in 12 of the 16 severe asthma cases. The FeNO and eosinophil levels in the nasal tissue, prior to the administration of omalizumab were predictors of the response to asthma treatment. **Conclusions:** These findings suggest heterogeneity among people with severe asthma. In addition, the phenotype associated with response to omalizumab, leading to improvement in asthma symptoms, comprises upper airway eosinophilia and high FeNO levels.

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Introduction

Asthma is a common and heterogeneous chronic disease, characterized by different clinical and pathophysiological profiles [1,2]. Inhaled corticosteroids (ICS) are used for controlling symptoms and decreasing cases of fatal bronchial asthma. However, approximately 5–10% of patients with asthma are classified as difficult-to-treat cases and exhibit persistent symptoms, despite treatment [3]. Omalizumab, a humanized monoclonal antibody for immunoglobulin E (IgE), is used in treating adult and infantile difficult-to-treat asthma, and is clinically effective in 60% of these patients [4].

The introduction of omalizumab decreased acute exacerbation and improved the peak expiratory flow rate or forced expiratory volume in 1 s (FEV₁) in patients with severe asthma [5,6]. In histology studies of

bronchial biopsy specimens from such patients, the infiltration of eosinophil and lymphocytes or the expression of IgE receptors has also been observed to decrease [7,8]. An accurate evaluation of the lower respiratory tract, based on histological analysis, is useful; however, it is not feasible in terms of the invasive procedure or medical cost in practice. Furthermore, from the theory of “one airway, one disease,” allergic airway inflammation in one site could extend to other sites in the respiratory tract [9]. For these reasons, nasal mucosa biopsy and brushing have been reported as a substitute for biopsy of the lower respiratory tract [10–12]. To date, no report exists examining the relationship between omalizumab and changes in histological findings in upper and lower airway inflammation.

In this study, we aimed to evaluate the efficacy of omalizumab in asthma treatment, based on the Global

Evaluation of Treatment Effectiveness (GETE). We also analyzed histological changes in the nasal mucosa, as well as the relationship between upper airway histology and lower airway inflammatory parameters (such as fraction of exhaled nitric oxide [FeNO] or sputum eosinophils), with asthma control in terms of the response to omalizumab.

Materials and methods

Study participants

The study participants included 16 patients (12 women and 4 men) diagnosed with severe asthma and perennial allergies, as determined by the radioallergen sorbent test (RAST). These patients were treated with omalizumab in combination with high dose ICS and a long-acting β -agonist at the Niigata University Medical and Dental Hospital from September 2011 to August 2015. The criteria for severe asthma have been reported previously [3]. Omalizumab doses were calculated, based on the total serum IgE levels and the patients' body weight, based on the manufacturer's instructions. This study was conducted in accordance with the Ethical Principles for Medical Research Involving Human Subjects, the Declaration of Helsinki, and with the approval of the Ethics Committee of Niigata University (approval no. 956). All participants provided written informed consent before enrollment.

Clinical assessments

Before omalizumab initiation, all participants underwent a physical examination; asthma control test (ACT; Japanese version); pulmonary function test; measurement of the FeNO level; peripheral blood eosinophil count; measurement of the sputum eosinophil percentage; measurement of total IgE level; RAST for perennial allergens such as mite, pet dander, or fungus; a computed tomography (CT) scan of the nose and paranasal sinuses; and a nasal biopsy under local anesthesia. After 16 weeks of treatment, the participants were examined by using the same parameters to evaluate omalizumab efficacy.

Pulmonary function tests were performed using a spirometer (SpiroSift SP-470; Fukuda Densi, Tokyo, Japan) in accordance with the American Thoracic Society (ATS) guidelines. The FeNO was measured using an nitric oxide analyzer (Kimoto Denshi, Osaka, Japan) with an online method, which was consistent with a previous mutual consensus statement from the ATS/European Respiratory Society [13]. Sputum induction and processing were performed as

previously described [14]. In brief, the participants received a procaterol (200 μ g) pretreatment and subsequently inhaled a hypertonic saline solution (3% w/v) delivered with an ultrasonic nebulizer for 15 min. Cell differentials were calculated as the percentage of cells in the total sputum. The diagnosis of chronic rhinosinusitis (CRS) was judged by otologists, based on symptoms and CT findings according to the Lund–Mackay score.

Histology and immunohistochemical staining

Nasal mucosa samples were fixed in 10% formalin and immersed in paraffin. Each section was cut to a thickness of 5 μ m, deparaffinized, and stained with hematoxylin and eosin (HE) staining for histological analysis. Immunohistochemical staining was achieved by using antibodies of cluster of differentiation (CD)3 (clone: EP449E), CD20 (clone: L26), tryptase (clone: AA1), Fc ϵ RI (clone: CRA1), IgE (goat polyclonal), and eosinophilic major basic protein (MBP; clone: BMK) according to the manufacturer's instructions. An anti-rabbit immunoglobulin G (IgG)-horseradish peroxidase-linked F(ab')₂ fragment (Amersham Pharmacia Biotech, Little Chalfont, UK) was used as the secondary antibody. After visualization with 3,3'-diaminobenzidine (Dojin Chemical, Kumamoto, Japan), the slides were counterstained with HE. Histological analyses were conducted as previously described [15]. Positive cells were counted in five randomly selected fields at 400 $\times$ magnification and expressed as the average of cell numbers in each of the fields.

Outcome measurement

Outcomes were primarily determined after 16 weeks of treatment via the GETE [16,17], based on a complete clinical evaluation of asthma symptoms that was based on all available information, including patient interviews and physical examinations. Treatment was rated as "excellent" (i.e. complete control of symptoms), "good" (i.e. marked improvement of symptoms), "moderate" (i.e. discernible but limited improvement of symptoms), "poor" (i.e. no appreciable change in symptoms), or "worsening of symptoms". Patients who positively responded to treatment (i.e. responders) had "excellent" and "good" GETE evaluations.

Statistical analysis

Mann–Whitney *U* tests were used to determine differences between groups. The correlation between FeNO

Table 1. Clinical characteristics and parameter changes before and after omalizumab treatment.

	Baseline	After omalizumab	<i>p</i> value
Subjects (<i>n</i>)		16	
Age (yr)		50 (28–81)	
Male (%)		25	
Disease duration (yr)		14 (2–70)	
ICS (fluticasone equivalent: $\mu\text{g}/\text{d}$)	800 (400–1200)	800 (400–1200)	1.00
OCS (prednisolone equivalent: mg/d)	0 (0–20)	0 (0–15)	0.25
SABA (times/wk)	7 (0–21)	0 (0–14)	<0.01*
ACT	16 (7–23)	20.5 (12–25)	0.01*
%FEV ₁	64.5 (24.3–104.9)	68.5 (35.4–106.9)	0.19
FEV ₁ /FVC	54.5 (30.6–89.9)	63.4 (31.6–87.5)	0.43
%MMEF	29.1 (7.3–102.7)	31.2 (9.9–105.8)	0.14
IgE (IU/L)	224 (16.4–1140)	480 (62.8–3590)	<0.01*
FeNO (ppb)	74 (8–421)	34.2 (7.3–311)	0.79
Sputum eos (%)	10 (0–89.5)	7.5 (0–74.3)	0.68
Blood eosinophils (/ μL)	289 (0–2118)	370 (0–3767)	0.64
Lund–Mackay score	4 (0–18)	5 (0–13)	0.94
CD3 (/F)	16.6 (2.6–35)	7.8 (0.4–33.8)	0.26
CD20 (/F)	2.6 (0–39.6)	5 (0–22.4)	0.91
MBP (/F)	3 (0–18)	2 (0–4)	0.01*
Tryptase (/F)	4.8 (0–14.6)	5.6 (0.2–20)	0.89
Fc ϵ RI (/F)	17.7 (1.2–43.2)	19.8 (1.0–57.4)	0.68
IgE (/F)	5.8 (0–22.4)	0.4 (0–16)	< 0.01*

ACT: asthma control test; FeNO: fraction of exhaled nitric oxide; Fc ϵ RI: high-affinity IgE receptor; %FEV₁: percent forced expiratory volume in 1 s; FVC: forced vital capacity; ICS: inhaled corticosteroid; MBP: major basic protein; %MMEF: percent maximum mid expiratory flow rate; OCS: oral corticosteroid; SABA: short-acting β -agonist. Data are expressed as the median (minimum–maximum). The asterisks (*) indicate a significant difference between the groups ($p < 0.05$).

or sputum eosinophils and the immunological staining of nasal mucosa was determined via Spearman rank analysis. Chi square tests were used for the categorical variables. Within-group (e.g., pre- and post-treatment) comparisons were conducted using Wilcoxon's signed-rank test. All pairs were compared using the Kruskal–Wallis test. All statistical analyses were performed with JMP software, version 11 (SAS Institute, Inc., Tokyo, Japan). For all statistical analyses, $p < 0.05$ was statistically significant.

Results

The baseline data of all 16 patients are shown in Table 1. Twelve patients were women, the median age of the patients was 50 years, and disease duration was 14 years. Fourteen (88%) patients used high doses of ICS ($\geq 800 \mu\text{g}/\text{day}$ equivalent to fluticasone) and six (38%) patients used oral corticosteroids. After initiating omalizumab, short-acting β -agonist (SABA) use and the ACT scores significantly improved ($p < 0.01$ and $p = 0.01$, respectively) and the levels of serum IgE significantly increased. However, IgE-positive cells in the nasal mucosa were significantly decreased. Moreover, the number of MBP-positive cells in the nasal mucosa was significantly decreased (Table 1). By contrast, after initiating omalizumab, no significant changes were observed for FeNO ($p = 0.79$) and blood eosinophils ($p = 0.64$). CRS diagnosed by otologists in this group was found in seven patients.

We compared the correlation between the levels of FeNO (Figure 1(A)) or sputum eosinophils (Figure 1(B)) and the immunological staining of the nasal mucosa. The number of cells positive for CD20 and MBP in the nasal mucosa was positively correlated with the value of FeNO (Figure 1(A)). Furthermore, the number of cells positive for CD20, MBP, tryptase, and IgE in the nasal mucosa was positively correlated with the percentages of sputum eosinophils (Figure 1(B)).

Based on the evaluation of GETE, we assessed 12 patients as responders, while the remaining four patients were nonresponders. We compared asthma symptoms, biomarkers, pulmonary function, and CT findings pre- and post-omalizumab in the treatment responders and nonresponders for asthma symptoms (Figure 2). Responders demonstrated significantly improved ACT scores and %FEV₁, as well as a significant reduction in SABA use ($p < 0.05$ for each value). This group had no significant changes in FeNO, sputum eosinophil, or Lund–Mackay scores. Nonresponders did not exhibit significant changes in any of these values.

Responders in asthma had significantly decreased percentages of CD3-, MBP-, and IgE-positive cells in their nasal mucosa after the initiation of omalizumab, compared with these percentages before treatment (Figure 3). No changes were seen in the percentages of CD20, tryptase, and the high-affinity IgE receptor (Fc ϵ RI) in the pre- and post-omalizumab samples

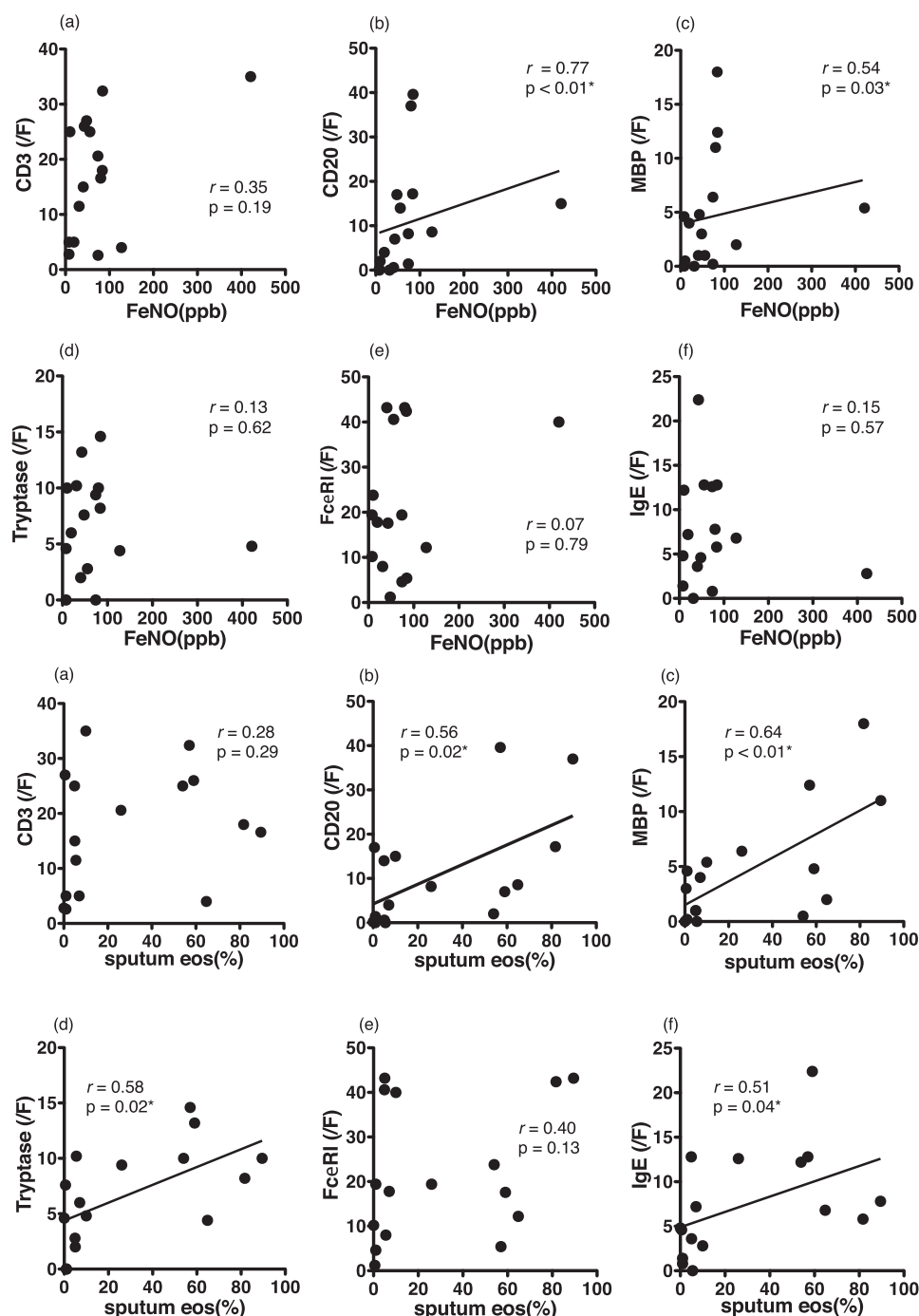


Figure 1 The correlation between fractional exhaled nitric oxide (FeNO) (A) or sputum eosinophil (B) and the immunological stain findings of the nasal mucosa: (a) CD3, (b) CD20, (c) major basic protein (MBP), (d) tryptase, (e) high-affinity immunoglobulin E receptor (FcεRI), and (f) immunoglobulin E (IgE). The r and p values were calculated via Spearman rank analysis. Asterisks indicate a significant difference between groups. $^*p < 0.05$. eos = eosinophil; ppb = parts per billion.

(Figure 3). The nonresponder group showed no significant changes between pre- and post-treatment.

To determine whether treatment responders had any specific characteristics affecting their response to omalizumab, we compared the baseline data of the responders and nonresponders (Table 2). Responders to asthma treatment had significantly higher levels of

FeNO and MBP-positive cells in their nasal mucosa than did the nonresponders.

Discussion

Omalizumab is used for treating cases of severe asthma that are resistant to conventional therapy; it

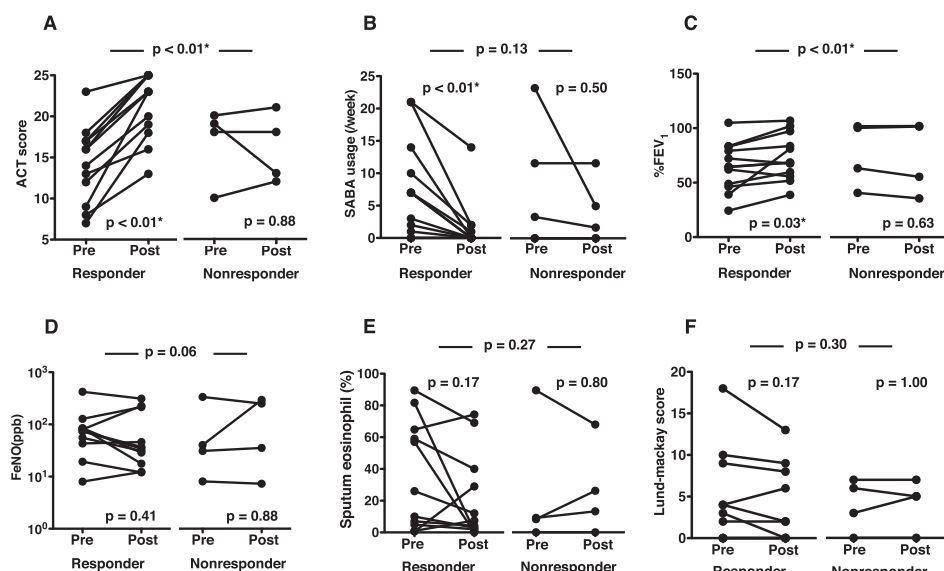


Figure 2 Changes in clinical parameters and biomarkers with omalizumab treatment. Values were obtained before (i.e. “Pre”) and 4 months after (i.e. “Post”) omalizumab treatment in responders and nonresponders in asthma symptoms. (A) ACT scores. (B) Short-acting β -agonist (SABA) frequency use per week. (C) Percent forced expiratory volume in 1 s (%FEV₁). (D) FeNO. (E) Sputum eosinophil percentage. (F) Lund–Mackay scores for nasal sinus CT scans. The p values were calculated via Wilcoxon matched-pairs signed rank tests.

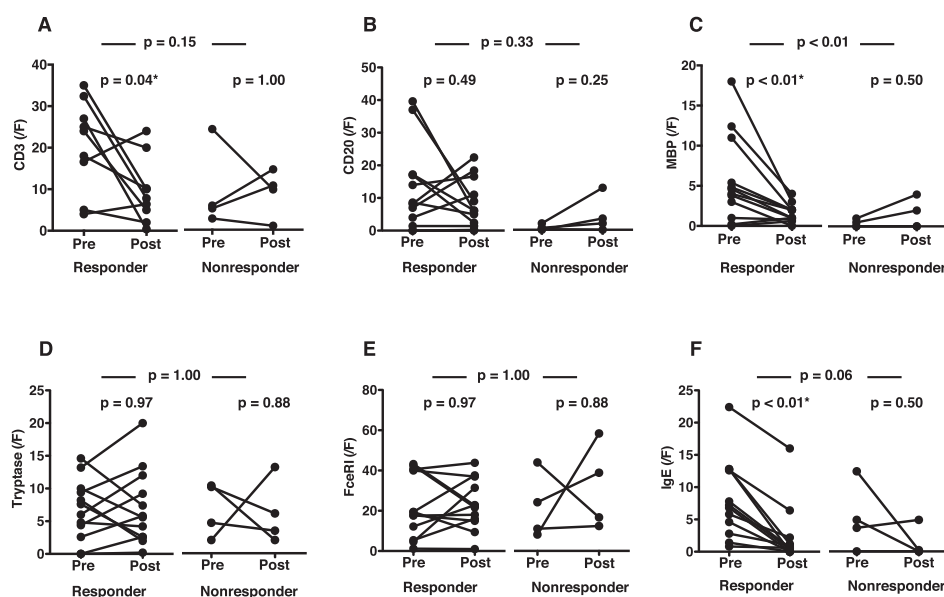


Figure 3 Changes in immunohistochemical staining pre- and post-treatment with omalizumab in asthmatic responders and nonresponders. (A) CD3. (B) CD20. (C) MBP. (D) tryptase. (E) Fc ϵ RI. (F) Human IgE. The p values were calculated via the Wilcoxon matched-pairs signed rank test.

has an efficiency rate of approximately 60% [4]. In our study, asthma symptoms were reduced in 75% of patients, and most responders exhibited a decrease in CD3- and MBP-positive cells in the nasal mucosa. High FeNO levels and nasal eosinophilia may be predictors of a positive response to omalizumab treatment for severe asthma.

The highlight of this study was the histological evaluation of the nasal mucosa instead of the bronchial

mucosa, and the correlation with FeNO or sputum eosinophils, which is considered the inflammatory assessment of the lower respiratory tract. As previously reported [10], MBP-positive cells in the nasal mucosa showed a positive correlation with FeNO and sputum eosinophils. Moreover, CD20-positive cells also showed a positive correlation with both, whereas tryptase- and IgE-positive cells showed a positive correlation with sputum eosinophils (Figure 1(A,B)). In a previous

Table 2. Clinical parameter summaries of the asthmatic responders and nonresponders.

	Responders (<i>n</i> = 12)	Nonresponders (<i>n</i> = 4)	<i>p</i>
Age (y)	62 (28–81)	50 (47–66)	0.86
Male patients (%)	25	25	1.00
Disease duration (y)	31.5 (2–70)	13.5 (10–18)	0.67
OCS usage (%)	33.3	50	0.60
ACT	14 (7–23)	18 (7–20)	0.28
%FEV ₁ (%)	64.4 (24.3–104.9)	81.4 (40.5–101.3)	0.59
%MMEF	25.6 (7.3–102.7)	35.2 (11.0–86.9)	0.86
IgE (IU/L)	128 (16.8–1140)	191 (16.4–443)	0.95
FeNO (ppb)	77 (8–421)	31.2 (8.1–40.5)	0.02*
Sputum eosinophil (%)	12.5 (0.8–89.5)	5.3 (0–54)	0.30
Blood eosinophil (/μL)	310.5 (0–2118)	137 (0–550)	0.48
Lund–Mackay score	4 (0–18)	4.5 (0–7)	1.00
CD3 (/F)	17.3 (2.6–35.0)	13.3 (2.8–25.0)	0.57
CD20 (/F)	7.6 (0.0–39.6)	0.4 (0.0–2.0)	0.06
MBP (/F)	4.3 (0.0–18.0)	0.3 (0.0–1.0)	0.04*
Tryptase (/F)	6.8 (0.0–14.6)	7.3 (2.0–10.2)	0.90
FcεRI (/F)	18.6 (1.2–43.2)	17.3 (8.0–43.2)	0.90
IgE (/F)	7.0 (0.8–22.4)	4.2 (0.0–12.2)	0.30

Data are expressed as the median (minimum–maximum). The asterisks (*) indicate a significant difference between the responders and nonresponders ($p < 0.05$).

ACT: asthma control test; FcεRI: high-affinity IgE receptor; FeNO: fraction of exhaled nitric oxide; %FEV₁: percent forced expiratory volume in one second; MBP: major basic protein; %MMEF: percent maximum mid expiratory flow rate; OCS: oral corticosteroid.

report [18], the nasal tryptase level reflected allergic inflammation of the respiratory tract, which was consistent with our findings.

A previous study [19] found that FeNO, blood eosinophils, and serum periostin were predictors of omalizumab treatment effects. In a recent report, the number of peripheral blood eosinophils was not important for reactivity to omalizumab [20]. In the current study, the FeNO level and MBP-positive cells in the nasal mucosa, but not the number of peripheral blood eosinophils and sputum eosinophil percentage, were associated with omalizumab efficacy in severe asthma. Based on the evidence that FeNO is strongly correlated with sputum eosinophils [21], it may be that eosinophilic inflammation in the upper and lower airways is important for a positive response to omalizumab; however, responders and nonresponders did not differ significantly in the sputum eosinophil percentage before or after omalizumab treatment. Some patients had received systemic corticosteroid treatment before the administration of omalizumab because of exacerbation, with a consequently decreased sputum eosinophil percentage at the start of treatment; although, at reevaluation after four months, this percentage had recovered. Furthermore, systemic corticosteroids might also reduce eosinophils and T-cells in the nasal mucosa; in some patients, the response to omalizumab did not correspond with the histological changes.

In this study, IgE-positive cells, but not FcεRI-positive cells, in the nasal mucosa were significantly decreased after treatment with omalizumab. The

reduction in free IgE is generally accompanied by a reduction in FcεRI expression on peripheral basophils and by decreasing cell responsiveness to allergens [22]. The expression of FcεRI in bronchial tissue [7] or in epidermal mast cells [23] decrease with omalizumab treatment. However, other reports have shown that the administration of omalizumab does not significantly reduce the expression of FcεRI in the nasal mucosa in people affected by seasonal allergic rhinitis, regardless of significant decreases in IgE expression [24]. Our data also showed that the expression of FcεRI is unlikely to correspond with the expression of IgE in nasal histology analyses.

This study has several limitations. The first limitation is that approximately 40% of patients using systemic steroids were included in this study. Hence, there is a possibility that biomarkers or histological findings associated with steroid therapy might be modified. In clinical practice, the uneven use of medications is inevitable, which can modify the outcomes of treatment. We had two patients who received a steroid burst just before the introduction of omalizumab. The use of these medicines in this study may be one reason the GETE evaluation was not correlated with an improvement in biomarkers such as FeNO and sputum eosinophil. However, the study result reflects actual clinical practice, which is considered sufficiently worth reporting.

The second limitation is that approximately 40% of patients with CRS were included. This is a matter of concern because of the possibility of histological differences between CRS and non-CRS cases. However,

with regard to this second limitation, there were no significant differences between CRS and non-CRS cases in the histological findings of the nasal mucosa (data not shown).

In summary, we investigated the efficacy of omalizumab in controlling severe asthma in a Japanese cohort. Among 16 patients treated with omalizumab, 12 patients effectively responded to the treatment in terms of asthma symptoms. When FeNO and the percentage of sputum eosinophils were used as indicators of lower airway inflammation, a positive correlation was found between CD20 B-cell, mast cell, and eosinophil counts in the nasal mucosa. Elevated FeNO and MBP-positive cells in the nasal mucosa before omalizumab administration were predictors of effective response to treatment in this study. These data suggest heterogeneity among individuals with severe asthma, and suggest that individuals with asthma who have a phenotype comprising eosinophilia in nasal mucosa and high FeNO levels may be responsive to omalizumab.

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