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Effect of Benralizumab on Mucus Plugs in Severe Eosinophilic Asthma

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Keywords
Benralizumab · Severe asthma · Mucus plug · Eosinophil cationic protein · Blood eosinophil

Abstract

Introduction: Mucus plugs are associated with airway obstruction in severe asthma and are involved in the formation of activated eosinophils. Benralizumab, an anti-interleukin-5 receptor antibody, markedly reduces not only peripheral blood eosinophils but also airway eosinophils; however, its effects on mucus plugs have not been clarified. In this study, we examined the efficacy of benralizumab on mucus plugs using computed tomography (CT) imaging. ***Methods:*** Twelve patients who were administered benralizumab and underwent CT before and approximately 4 months after the introduction of benralizumab were included in this study, and the number of mucus plugs before and after benralizumab administration was compared. The correlation between the clinical background and treatment effect was also examined. ***Results:*** The number of mucus plugs significantly decreased after the introduction of benralizumab. The number of mucus plugs was correlated with sputum eosinophil percentage and eosinophil cationic protein in the sputum supernatants and inversely correlated with forced expiratory volume in 1 s (FEV_1). Benralizumab induction resulted in a marked decrease in blood and sputum eosinophil levels and a significant improvement in asthma symptoms, quality of life scores, FEV_1, and exacerbation frequency. Furthermore, there was a significant correlation between the reduction in mucus plugs and changes in the symptom score or FEV_1. ***Discussion/Conclusion:*** These data suggest that benralizumab may have the potential to improve symptoms and respiratory function in patients with severe eosinophilic asthma by reducing mucus plugs.

Introduction

Severe asthma accounts for approximately 5–10% of all asthma patients [1, 2]. Clinical problems of severe asthma include a high frequency of exacerbations and a significant decline in respiratory function over time, particularly the forced expiratory volume in 1 s (FEV_1) [3, 4]. Airway remodeling caused by chronic airway inflammation is an important factor in the mechanism of obstructive impairment [5]. However, other pathologies, such as airway obstruction associated with mucus plugs, have also

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been revealed by imaging analyses such as multidetector computed tomography (MDCT).

Dunican et al. [6] reported that the number of mucus plugs based on the visual assessment of MDCT lung images in severe asthma revealed a negative correlation with FEV_1 and a positive correlation with sputum eosinophils. Moreover, several reports have analyzed mucus plugging in chronic airway diseases such as asthma and chronic obstructive pulmonary disease with MDCT or regional ventilation defects using magnetic resonance imaging [7–9]. In studies that monitored the number of mucus plugs in severe asthma patients, those with mucus plugs may have had obstructive impairment owing to the presence of mucus plugs for 3 years [10]. Furthermore, since airway eosinophils are involved in mucus plug formation, biologics targeting eosinophils are presumed to be promising agents for reducing mucus plugs.

Benralizumab, an anti-interleukin-5 (IL-5) receptor alpha-chain antibody, is a biologic agent that is expected to reduce exacerbations and improve FEV_1 by suppressing eosinophilic airway inflammation associated with definite eosinophil clearance [11–14]. We have previously examined the efficacy of clinical parameters and airway thickness using MDCT after benralizumab induction. Most participants demonstrated a significant improvement in clinical parameters, a significant decrease in right B^1 airway wall thickness, and an increase in the number of bronchi visible on computed tomography (CT) [15]. However, the association between benralizumab and changes in mucus plugs and sputum eosinophils has not yet been analyzed. In this study, we examined mucus plug count using MDCT analysis in 12 cases before and after administering benralizumab.

Materials and Methods

Study Participants

The study involved 12 patients (six women and six men) diagnosed with severe uncontrolled eosinophilic asthma, according to the American Thoracic Society/European Respiratory Society (ATS/ERS) criteria [1]. These patients were treated with benralizumab and underwent CT before and 4–6 months after the start of benralizumab treatment at Niigata University Medical and Dental Hospital from June 2018 to October 2021. Cases in which CT could not confirm the presence of mucus plugs before treatment, or cases with other diseases resulting in mucus plugs, such as allergic bronchopulmonary aspergillosis (ABPA) or chronic airway infection, were excluded from this study. This study was conducted in accordance with the ethical principles for medical research involving human subjects, the Declaration of Helsinki, and received the approval of the Ethics Committee of Niigata University (approval no. 2524). All participants provided written informed consent prior to enrollment.

Clinical Assessments

Before the initiation of benralizumab, all participants underwent a physical examination, and the following parameters were examined: asthma control test (ACT; Japanese version), asthma quality of life questionnaire (AQLQ; Japanese version), pulmonary function test, measurement of the fraction of exhaled nitric oxide (FeNO) level, peripheral blood eosinophil count, sputum eosinophil percentage, and measurement of total immunoglobulin E (IgE) levels. After 4 months of treatment with benralizumab, the participants were examined using the same parameters to assess the efficacy of benralizumab. We also evaluated asthma exacerbations, identified by the use of a systemic corticosteroid due to asthma symptoms 12 months before and after the administration of benralizumab.

Pulmonary function tests were performed using a spirometer (SpiroSift SP-470; Fukuda Denshi Co. Ltd. Tokyo, Japan), according to the ATS guidelines [16]. FeNO levels were measured using a nitric oxide analyzer (NIOX VERO® Circassia AB, Uppsala, Sweden) in accordance with a previous mutual consensus statement by the ATS/ERS [17]. Sputum induction and processing were performed as previously described [18]. Cell differentiation was analyzed on May Giemsa-stained cytospin preparations and expressed as the percentage of nonsquamous cells. Eosinophil cationic protein (ECP) in the supernatants was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemical, Ann Arbor, MI, USA).

Chest CT and Mucus Plug Analysis

Multidetector-row CT (MDCT) imaging was performed mainly using an MDCT machine (SOMATOM Force; Siemens, Munich, Germany). All images were obtained in the supine position during full inspiration breath-hold. Images were reconstructed using slice thickness at 1 mm slice intervals. The analysis of mucus plugs was performed by two respiratory physicians blinded to the patients' data (NS and YM). Airway mucus plugs were identified and quantified using a previously reported scoring system [6, 9]. Briefly, mucus plugs were defined as complete occlusion of the airway [6, 7, 9]. The lung zone within 2 cm from the costal or diaphragmatic pleura was excluded because the airways in that zone were too small to ascertain complete occlusion by luminal plugs. A mucus score of 0 or 1 based on the absence or presence of mucus plugs was generated for each CT scan as an aggregation of the number of bronchopulmonary segments with luminal plugging, ranging from 1 to 18.

Statistical Analysis

Data are expressed as median (minimum-maximum). Mann-Whitney U tests were used to evaluate the differences between the two groups. Within-group (pre- and post-treatment) comparisons were conducted using Wilcoxon's signed-rank test. The correlation between the morphological findings and clinical parameters was determined using Spearman's rank analysis. All statistical analyses except for post hoc power calculation were performed using JMP software version 11 (SAS Institute, Inc., Tokyo, Japan). A post hoc power calculation was performed using G*Power software version 3.1 (download from http://www.gpower.hhu.de/). Statistical significance was set at $p < 0.05$ for all statistical analyses.

Table 1. Summary of clinical parameters of the subjects

Cases (*n*)	12
Male (%)	6 (50)
Age (years)	66 (31–77)
Duration (from diagnosis) (y)	20.5 (9–48)
BMI (kg/m2)	23.9 (21.0–32.0)
Smoking, *n* (%)	
Never smoked	9 (75.0)
Past history of smoking	3 (25.0)
CRS comorbidity	8 (66.7)
EGPA comorbidity	1 (8.3)
ICS (μg/day)	900 (400–1000)
OCS daily	3 (25.0)
Previous biologics	
Omalizumab	2
Mepolizumab	2
Exacerbation (/year)	5 (0–24)
ACT	17 (8–25)
AQLQ	4.7 (2.7–5.8)
Blood eosinophil (/μL)	735 (41–1630)
IgE (U/L)	697 (24–4610)
FeNO (ppb)	100 (21–274)
%FEV1	69.1 (32.7–102.1)
FEV1/FVC (%)	61.6 (28.6–80.8)
Mucus plug	5.5 (1–12)
Sputum eosinophil (%)	40.3 (23–73.5)
Sputum ECP (mg/mL)	36.3 (11.3–86.0)
Time from CT scan to benralizumab initiation (days)	28 (0–208)
Time from benralizumab initiation to second CT (days)	113 (111–182)
Time of evaluation from benralizumab initiation (days)	112 (111—126)
Total follow-up days from benralizumab initiation (days)	1,085 (504—1793)

Data are expressed as number (percentage) or median (minimum-maximum). BMI, body mass index; CRS, chronic rhinosinusitis; EGPA, eosinophilic granulomatosis with polyangiitis; ICS, inhaled corticosteroid; OCS, oral corticosteroid; ACT, asthma control test; AQLQ, asthma quality of life questionnaire; IgE, immunoglobulin E; FeNO, fraction of exhaled nitric oxide; $\%FEV_1$, percent forced expiratory volume in 1 s; FVC, forced vital capacity; ECP, eosinophil cationic protein.

Results

Originally, the total number of patients who took CT scan before and 4–6 months after benralizumab was 17. However, sputum samples were not collected from 3 patients, 1 patient had nontuberculous mycobacterium as comorbidity, and CT scan before benralizumab did not show any mucus plugs in 1 patient; therefore, these 5 patients were excluded from this study analysis (online suppl. figure; see online suppl. material at www.karger.com/doi/10.1159/000530392). The baseline data for all 12 patients are presented in Table 1. Six patients were male, the median age was 66 years, and the disease duration was approximately 20 years. Eight patients (67%) had chronic rhinosinusitis and one (8.3%) had eosinophilic granulomatosis with polyangiitis as comorbidities. The median inhaled corticosteroids dose was 900 μg/day (equivalent to fluticasone), and 3 patients (25%) received maintenance oral corticosteroids. The median frequency of exacerbations in the previous year, defined as a systemic corticosteroid burst due to asthma symptoms, was five. The baseline median ACT and AQLQ scores were 17 and 4.7, respectively. The baseline blood eosinophil count was 735 per μL. Median IgE and FeNO levels were 697 U/L and 100 ppb, respectively. Most patients showed obstructive impairment based on pulmonary function data. The baseline mucus plug count was 5.5, whereas the percentages of sputum eosinophils and sputum ECP concentrations were 40.3% and 36.3 mg/mL, respectively. The median time from the first CT scan to the start of benralizumab and time from the start of benralizumab to second CT were 28 and

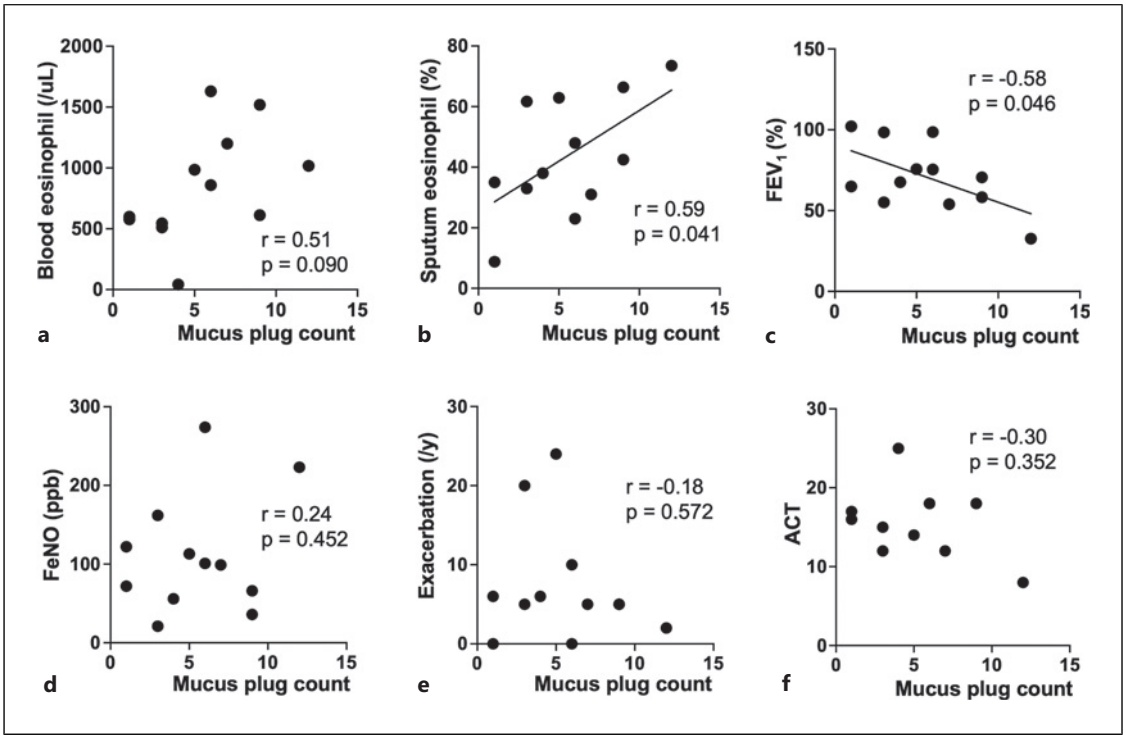

Fig. 1. Correlation between mucus plug count and each parameter. **a** Blood eosinophil count. **b** Percentage of sputum eosinophil. **c** %FEV1. **d** FeNO levels. **e** Exacerbation counts in previous year. **f** ACT score. The *r* and *p* values were calculated using Spearman's rank analysis. FEV1, percent forced expiratory volume in 1 s; FeNO, fraction of exhaled nitric oxide; ACT, asthma control test.

113 days, respectively (Table 1). The median time of evaluation of clinical parameters except for exacerbation frequency was 112 days (Table 1).

The mucus plug count was analyzed in relation to the baseline data. Mucus plug count showed a significant positive correlation with sputum eosinophil percentage (shown in Fig. 1b; 1-β = 0.6) and a significant negative correlation with $\%FEV_1$ (shown in Fig. 1c; 1-β = 0.6). In addition, peripheral blood eosinophil count, FeNO, number of exacerbations in the previous year, and ACT were analyzed in relation to mucus plug count, but no significant correlation was found (shown in Fig. 1a, d, e, and f). Regarding ECP in the sputum, the levels of ECP in sputum supernatants were positively correlated with sputum eosinophil percentage and mucus plug count (shown in Fig. 2a and b; sputum eosinophil: 1-β = 0.97, mucus plug count: 1-β = 0.7).

Changes in the data 4 months after the initiation of benralizumab are shown in Table 2. There was a significant improvement in ACT and AQLQ. There was also a marked decrease in the number of exacerbations. In addition to eosinophils in the peripheral blood, there was a significant decrease in sputum eosinophil percentage and sputum ECP concentration. The values of $\%FEV_1$ and FEV_1/FVC in respiratory function also showed significant improvement. In contrast, serum IgE (1-β = 0.11) and FeNO levels (1-β = 0.16) showed no significant changes.

Mucus plug count decreased significantly after benralizumab treatment. The groups could be divided into those in which the mucus plug count decreased by more than 50% and those where it was less than 50%. The former group with decreased mucus plugs was characterized by a higher sputum eosinophil percentage and ECP levels in the sputum supernatants at baseline (shown in Fig. 3b, c).

We analyzed whether changes in the mucus plug count correlated with changes in clinical parameters. A positive correlation was observed between changes in mucus plug count and changes in $\%FEV_1$ and ACT scores (shown in Fig. 4a, b; $\%FEV_1$: 1-β = 0.75, ACT: 1-β = 0.85), whereas there was no significant correlation between changes in mucus plug and changes in blood eosinophil count or sputum eosinophil percentage (shown in Fig. 4c, d; blood

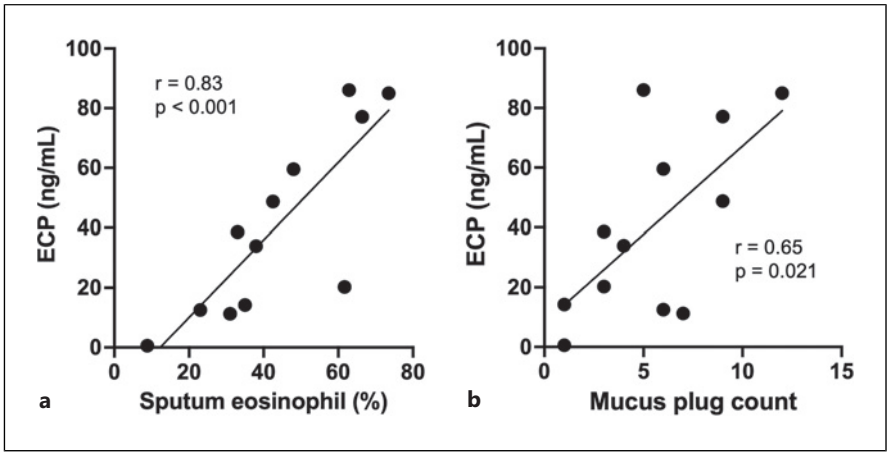

Fig. 2. Relationship between ECP in sputum supernatant and percentage of sputum eosinophil and mucus plug count. **a** Percentage of sputum eosinophil. **b** Mucus plug count. The *r* and *p* values were calculated using Spearman's rank analysis. ECP, eosinophil cationic protein.

Table 2. Changes in clinical parameters and biomarkers after benralizumab treatment

	Pre	Post	*p* value
ACT	17 (8–25)	23 (18–25)	0.001
AQLQ	4.7 (2.7–5.8)	6.0 (4.06.8)	<0.001
Exacerbation (/year)	5 (0–24)	0 (0–8)	<0.001
Blood eosinophil (/μL)	735 (411630)	0 (0—)	<0.001
IgE (U/L)	697 (24–4610)	665.5 (35.4–4070)	0.608
FeNO (ppb)	100 (21–274)	55 (15–300)	0.470
%FEV_1	69.1 (32.7–102.1)	82.8 (54.8–113.6)	<0.001
FEV_1/FVC (%)	61.6 (28.6–80.8)	68.4 (43.8–82.9)	0.019
Mucus plug	5.5 (1–12)	1 (0–7)	0.001
Sputum eosinophil (%)	40.3 (23–73.5)	0.0 (0.0–1.0)	<0.001
Sputum ECP (mg/mL)	36.3 (11.3–86.0)	9.3 (1.8–37.2)	0.015

Data are expressed as number (percentage) or median (minimum-maximum). Pre, before benralizumab treatment; post, 4 months after benralizumab treatment; ACT, asthma control test; AQLQ, asthma quality of life questionnaire; IgE, immunoglobulin E; FeNO, fraction of exhaled nitric oxide; %FEV_1, percent forced expiratory volume in 1 s; FVC, forced vital capacity; ECP, eosinophil cationic protein.

eosinophil: 1-β = 0.38, sputum eosinophil: 1-β = 0.26). Furthermore, changes in the AQLQ score or exacerbation frequency were not correlated with changes in the mucus plug count (data not shown).

Discussion

In the present study, 12 patients with severe eosinophilic asthma were treated with benralizumab. Mucus plug count on CT was evaluated before and after treatment. Our study revealed two major novel findings: (1) benralizumab reduced mucus plug count, and (2) the degree of amelioration of mucus plug was associated with the baseline sputum eosinophil percentage and sputum ECP levels. To the best of our knowledge, this is the first case series report on changes in mucus plugs and sputum eosinophils in patients treated with benralizumab.

Concerning the relationship between benralizumab and mucus plugs, Hearn et al. [19] reported that benralizumab was effective in severe eosinophilic asthma with or without mucus plugs and that changes in clinical symptoms, FEV_1, annual exacerbation rate, and maintenance oral corticosteroid usage were not related to the presence of mucus plugs. Case reports have also shown that benralizumab is effective in improving mucus plugs in patients with ABPA [20] and eosinophilic bronchiolitis [21], both of which are thought to be related to extracellular trap cell death (ETosis). Furthermore, a report analyzing mucus plugs by CT and ventilation defect percentage (VDP) by ^{129}Xe magnetic resonance imaging before and after a single dose of benralizumab [22]

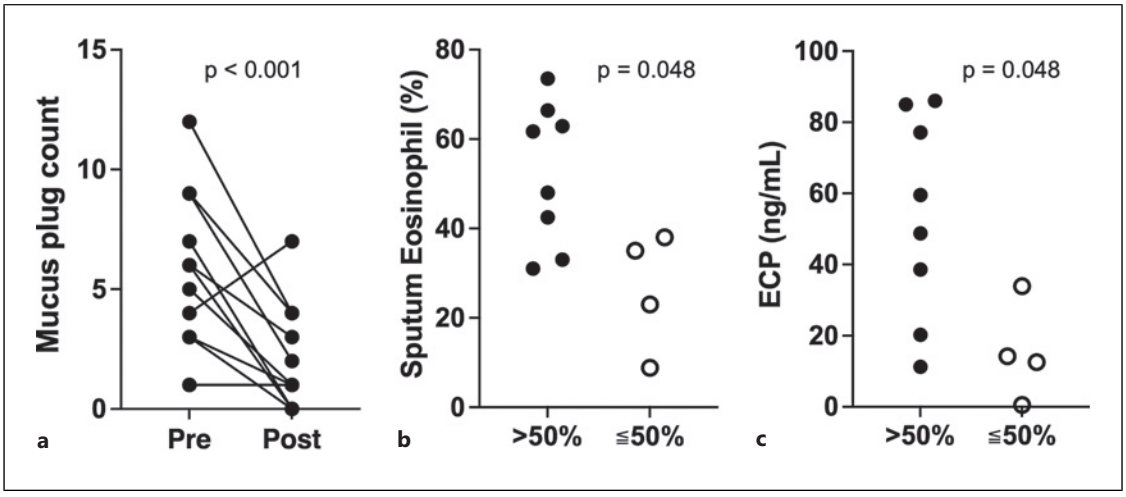

Fig. 3. Change in mucus plug count and response for mucus plug count before and after benralizumab. **a** Changes in the mucus plug count in CT with benralizumab treatment. Values were obtained before ("pre") and 4 months after ("post") benralizumab treatment. The patients treated with benralizumab were divided into two groups: those with >50% reduction in mucus plug count (>50%) and those with <50% reduction (≤50%), and the percentages of sputum eosinophil (**b**) and ECP (**c**) in sputum supernatant in the baseline were compared. The *p* values were calculated via Wilcoxon matched-pairs signed-rank tests (**a**) and Mann-Whitney U tests (**b**, **c**). ECP, eosinophil cationic protein.

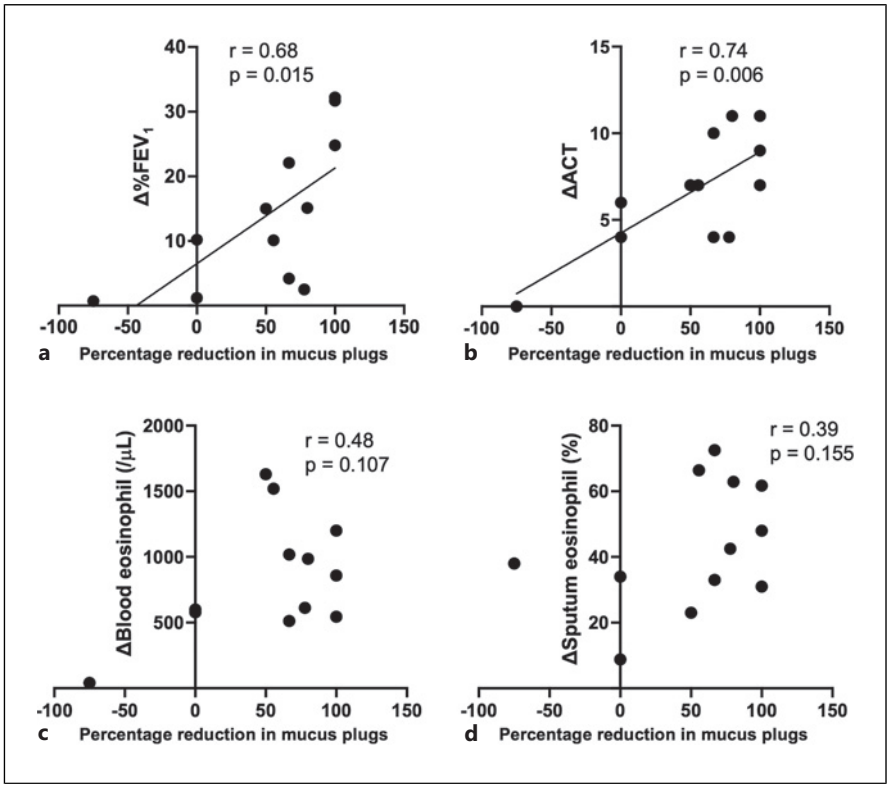

Fig. 4. Changes in mucus plug counts correlated with the changes in clinical parameters. A positive correlation was observed between the changes in mucus plug counts and the changes in $\%FEV_1$ (**a**) and ACT score (**b**). A nonsignificant correlation occurred between the changes in blood eosinophil (**c**) and the changes in sputum eosinophil percentage (**d**). The *r* and *p* values were calculated using Spearman's rank analysis. FEV_1, percent forced expiratory volume in 1 s; ACT, asthma control test.

showed a significant correlation between mucus plug count and VDP. In addition, a significant improvement in VDP was observed in patients with a higher mucus plug count, suggesting that mucus plug has a direct effect on ventilation defects, in agreement with another report [8]. In this study, amelioration of mucus plugs was

associated with improvements in FEV_1 and ACT. Furthermore, eosinophils in the airways are closely linked to this improvement. The results may represent the characteristics of benralizumab, which is highly effective in reducing eosinophil counts in the airways [11, 23].

The effects of other biologics on mucus plugs have also been reported. Dupilumab, an anti-IL-4 receptor antibody, has been used as an alternative therapy to systemic steroids and mepolizumab, an anti-interleukin-5 antibody, produces marked improvement in chest shadows, including mucus plugs [24–26]. Benralizumab eliminates activated eosinophils, which suppress eosinophil granule protein and ETosis, thus reducing mucus plugs; however, it is speculated that dupilumab reduces mucus production by suppressing the biological activity of IL-13 and expression of MUC5AC in goblet cells, which is essential for mucus production, resulting in a clinical effect [27, 28].

In this study, we observed a correlation between sputum eosinophil percentage, ECP of sputum supernatants, and the mucus plug count. Duncan et al. [6] found a correlation between mucus plug count and the levels of eosinophilic peroxidase (EPO) in sputum supernatants and described that EPO-generated oxidants were involved in mucus plug formation. An ELISA kit for EPO was also used in this study; however, we could not measure all cases due to sample volume issues. No significant correlation was observed between sputum eosinophil percentage and mucus plug count (data not shown). Although technical issues such as specimen preparation were considered [29], given the strong correlation between sputum eosinophil percentage and ECP levels of sputum supernatants, we speculated that EPO was also present in parallel with ECP [30]. In addition, fibrin formation associated with the release of tissue factors from eosinophils [31] and extracellular trap cell death (ETosis)-induced debris, as observed in ABPA [32], are also considered mechanisms for mucus plug formation.

A correlation between the reduction rate of the mucus plug count and changes in FEV_1 and ACT was observed in this study. In a 3-year observational study of SARP-3 [10], changes in the mucus plug count were reported to correlate with changes in lung function indices, including FEV_1, which may support the present study. Increases in FEV_1 have been reported in clinical studies of benralizumab [12–14, 33]; however, the underlying mechanism is unclear. Based on our data, reduction in mucus plugs might be a mechanism of FEV_1 improvement. In cases of severe eosinophilic asthma, confirmation of sputum eosinophil and mucus plugs may be predictive of FEV_1 improvement with benralizumab.

In this study, the number of mucus plugs increased in 1 patient but remained unchanged in 2 patients despite a decrease in sputum eosinophils. In particular, the patient with an increased number of mucus plugs had an underlying eosinophilic granulomatosis with polyangiitis as a comorbidity. This patient had long-term history of oral steroid usage and it is possible that other pathologies besides eosinophilic inflammation such as infection were also present.

Because this study included a small sample size of 12 patients, it is possible that a false-negative type 2 error could have occurred. The post hoc power calculations (1-β) showed that some were insufficient, whereas the recommended power is expected to be around 0.8 [34]. In particular, an association between peripheral blood eosinophils and mucus plugs could be significant if the sample size increases. Furthermore, this study found a correlation between clinical symptoms (ACT) or FEV_1 improvement and mucus plug reduction; however, other effects of benralizumab, such as airway wall changes [15, 35] and decreased airway hyperresponsiveness [36], may also be involved. A larger sample size of benralizumab use should be analyzed in future studies.

This study had several limitations. First, this was a single-arm study without controlled comparison, and the sample size was small owing to the single-center nature of the study. Second, the timing of the first CT scan was different because of real clinical data. In particular, in some cases, the scans were taken about 6 months prior. Third, the percentage of sputum eosinophils was high in most cases. In a practical setting, benralizumab is selected in cases with expected efficacy, that is, those with high blood eosinophil counts or high sputum eosinophil percentage, thus resulting in case bias. Different mucus plugs have been reported to exist for different airway inflammation types, and there are pathologies associated with smoking and eosinophilic airway inflammation [37]. The effect of benralizumab on mucus plugs in patients with less eosinophilic inflammation is also an intriguing prospect. Finally, this study was evaluated after 4 months (CT after 4–6 months), except for exacerbations. Thus, it may not reflect the seasonal worsening of symptoms or airway inflammation fluctuation (e.g., FeNO).

In summary, we examined the efficacy of benralizumab on mucus plugs using CT imaging. Twelve patients were studied, and the number of mucus plugs decreased significantly after benralizumab treatment. The number of mucus plugs was correlated with sputum eosinophil percentage and ECP of sputum supernatants and inversely correlated with FEV_1. Furthermore, there was a significant correlation between the rate of mucus plug reduction and changes in symptom score or FEV_1 in this study. The presence of mucus plugs and peripheral blood

eosinophil counts was considered a potential predictor of treatment when selecting biologics.

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Statement of Ethics

This study was conducted in accordance with the ethical principles for medical research involving human subjects and the Declaration of Helsinki and was approved by the Ethics Committee of Niigata University (approval no. 2524). All participants provided written informed consent prior to enrollment.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Natsumi Sakai and Toshiyuki Koya designed the study and prepared the manuscript. Natsumi Sakai, Toshiyuki Koya, Yui Murai, Fumito Tsubokawa, Kentaro Tanaka, Shun Naramoto, Ami Aoki, Kenjiro Shima, and Yosuke Kimura contributed to the data collection. Toshiyuki Koya, Satoshi Watanabe, Takashi Hasegawa, and Toshiaki Kikuchi performed statistical analysis and interpretation of the results. All the authors have read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding authors.

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