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Running title: NP eosinophilia predicts asthma symptoms after ESS

Nasal polyp eosinophilia and FeNO may predict asthma symptoms development after endoscopic sinus surgery in CRS patients without asthma

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Abstract

Background: Asthma is a significant comorbidity of eosinophilic chronic rhinosinusitis (CRS). Type2-driven biomarkers such as sinus tissue eosinophilia and fractional nitric oxide (FeNO) may be utilized to detect high risk patients who develop asthma symptoms after endoscopic sinus surgery (ESS) in CRS patients.

Methods: Thirty-six CRS patients without asthma who agreed to undergo ESS between October 2015 and December 2017 were prospectively observed for 12 months following ESS. They were monitored for the development of typical asthma symptoms including dyspnea, wheezes, and cough which responded to anti-asthma medication. Biomarkers were compared between patients who developed asthma symptoms after ESS (asthma symptoms group) and those who did not (non-asthma group). Biomarker changes following ESS intervention were also evaluated.

Results: Six patients were lost to follow after ESS. Thus, 30 CRS patients [16 with nasal polyps (NPs) proved by surgery] were followed. Seven (23%) newly complained of asthma symptoms during follow-up. Levels of FeNO and the prevalence of eosinophilic NPs (eosinophils ≥ 70 /high power fields) were significantly higher in the asthma symptom group than in non-asthma group [50.7 ppb vs 22.4 ppb for FeNO levels, and 100% ($n = 3$) vs 23% ($n = 3$) for eosinophilic NP prevalence, both $p < 0.05$]. Levels of sputum periostin decreased significantly by ESS in the non-asthma group. However, changes of biomarkers after ESS were comparable between the two groups.

Conclusions: Eosinophils in NPs (≥ 70 /high power fields) and preoperative FeNO may be significant biomarkers for predicting the development of asthma symptoms after ESS.

Keywords Chronic rhinosinusitis, Asthma, Nasal polyps

Abbreviations:

CRS: chronic rhinosinusitis, ESS: endoscopic sinus surgery, FeNO: fractional nitric oxide, IL: interleukin, MS: Lund–Mackay score, HPF: high power field, QoL: quality of life, NPs: nasal polyps, SNOT-22: Sino-nasal Outcome Test-22

Introduction

The prevalence of asthma has been increasing worldwide(1). The European Community Respiratory Health Survey revealed that nasal allergy is a significant risk factor for the adult onset of asthma, together with female gender, the presence of airway hyperresponsiveness and atopic predisposition, reduced expiratory volume in one second (FEV_1), and maternal asthma(2). Another epidemiological survey in Europe, the GA²LEN study, has subsequently demonstrated that approximately 20–60% of patients with chronic rhinosinusitis (CRS) with nasal polyps (CRSwNPs) have asthma(3). This indicates that CRSwNP and asthma may share common pathogenetic features. These upper and lower airway conditions are functionally and immunologically associated(4, 5), and often called “unified airway diseases”.

CRS is subdivided into the two pathologic features; eosinophilic and non-eosinophilic CRS(6). Asthma is a well-known comorbidity of eosinophilic CRS often complicated by nasal polyps (NPs) and sensitization to *Staphylococcus aureus* enterotoxins (SEs)(6–8). Endoscopic sinus surgery (ESS) is a common treatment for eosinophilic CRS where patients are refractory to medical treatments including topical nasal corticosteroids, although there is a high rate of recurrence following polyp removal(6, 9, 10). In particular, eosinophilic NPs are strongly associated with the presence of asthma(6, 7), its recurrence after ESS(11), and type2-predominant lower airway inflammation(12). This suggests that severe eosinophilic CRS patients who may require ESS are at the greatest risk for developing asthma symptoms in the near future. However, it remains unclear whether tissue eosinophilia in NPs may be associated with the development of asthma symptoms after ESS in such patients.

The aim of this study is to determine the biomarkers predicting those CRS patients who are at high risk of developing asthma symptoms after ESS. We hypothesized that tissue eosinophilia and type2-driven systemic/lower airway biomarkers (i.e. blood eosinophils, serum total immunoglobulin E and periostin, and FeNO) could potentially be such predictors. In this study, we limited enrollment of patients to those who are going to undergo ESS as these individuals may have more intense upper airway type2 inflammation which is available for pathologic investigation, together with systemic/lower airway type2 inflammation, and therefore may be at higher risk of developing asthma symptoms.

Materials and Methods

Subjects

This study is a *post hoc* analysis of our previous study which evaluated the pathophysiological link between upper and lower airways in CRS patients(12). We prospectively recruited CRS patients without asthma ($n = 36$) and those with comorbid asthma ($n = 20$) who visited our hospital to undergo ESS between October 2015 and December 2017. All patients had already received medical interventions such as macrolide antibiotics, non-sedative anti-histamine1 receptor antagonists, and nasal corticosteroids for at least three months before enrolling the study. Thus, ESS was considered, indicated, and recommended by otorhinolaryngology specialists to improve their condition. Among the 56 CRS patients enrolled, 36 without asthma symptoms at enrollment were prospectively followed for 12 months following ESS and analyzed in this study (Figure 1). All medications taken for CRS were continued for six months following ESS. Written informed consent was obtained from all participants. Patients suffering from other obvious respiratory disease, and those who withdrew informed consent or did not complete the follow-up examination were excluded from the study.

This study was approved by the Ethics Committee of Nagoya City University (Number 1165) and was registered on the UMIN Clinical Trials Registry (Registry ID UMIN000018672).

Laboratory tests

Patients underwent a diagnostic work-up, including examination by asthma specialists (Y.K., M.T., and A.N.) and an otorhinolaryngology specialist (M.S.) at both enrollment and 12 months after ESS. Detailed information has been published previously(12).

We investigated whether biomarkers such as blood and sputum eosinophil counts, serum total immunoglobulin E, serum specific immunoglobulin E against SEs (SEA and SEB), and serum and sputum periostin levels are useful for detecting high risk patients who develop asthma symptoms after ESS. Patients were considered to be sensitized against SEs if they showed a titer of specific immunoglobulin E against either SEA or SEB of ≥ 0.10 UA/ml, as previously reported(13). Serum and sputum periostin levels were measured using an ELISA at Shino-Test (Kanagawa, Japan) as described previously(12, 14–16).

Clinical, physiological and radiological measurements

Lung function, radiological severity of CRS, olfactory function, and FeNO were assessed both before and 12 months after ESS. If patients complained of new typical asthma symptoms (dyspnea, wheezing, chest tightness, and cough, which were variable) with or without audible wheezes by auscultation during the follow-up, FeNO was also measured at that time. FEV₁ was measured according to the American Thoracic Society/European Respiratory Society recommendations using Chestac-8900 (Chest, Tokyo, Japan)(17). Airway hyperresponsiveness to inhaled methacholine was also evaluated using Astograph (Jupiter-21, Chest, Tokyo, Japan) prior to ESS(18, 19). Ten doubling concentrations of methacholine (49 to 25 000 μ g/mL) were inhaled for 1 min each in ascending order until either the maximum concentration was reached or respiratory resistance increased to two folds of baseline values, as determined during 1 min inhalation of saline before the initiation of methacholine. Cumulative dose of methacholine (mg/mL) is represented by units, where one unit equals 1.0 mg/ml of inhaled methacholine(18). Airway hyperresponsiveness was considered to be present if Dmin, an index of airway sensitivity, was ≤ 12.5 units (the total cumulative dose of methacholine ≤ 12.5 mg/mL)(19). The Lund–Mackay system is a well utilized method of evaluating radiological severity of CRS(20, 21), in which opacification in the 5 sinus cavities (maxillary, anterior ethmoid, posterior ethmoid, sphenoid and frontal sinuses) and the ostiomeatal complex of both sides is rated. Scores range from 0 to 2 in each region, and are summed to calculate the total score, the maximum being 24. Higher scores indicate more severe CRS (i.e. 0 = complete lucency, 24 = complete opacity of all the sinuses). All scoring was made by a radiology specialist (Y.O.) in a blinded manner. The ‘Open Essence’ method was adopted to determine olfactory function(22). Consisting of 12 cards with different odorants (e.g. condensed milk, cooking gas, curry, cypress wood, rose, sweaty-smelling clothes, perfume and roasted

garlic), the number of correctly identified smells is summed to determine the total score (ranging from 0 to 12). A higher score indicates better olfactory function. FeNO levels were measured at an expiratory flow rate of 50 ml/s using a Sievers NOA 280i chemiluminescence analyser (GE Analytical Instruments, Boulder, USA) according to current guidelines(23).

The SNOT-22 questionnaire to assess nasal and paranasal sinus related quality of life (QoL) was also completed. SNOT-22 is a validated questionnaire consisting of 22 items regarding symptoms of CRS, each scored on a Likert scale ranging from 0 to 5 points. The total score is 0 to 110 points with higher scores representing the worse sinus-related QoL(24, 25). We obtained permission to use the questionnaire for this study from Prof Jay Piccirillo, Washington University.

Histological evaluation of ESS samples

Inflamed sinus tissue samples were taken from 34 patients under general anesthesia by otorhinolaryngology specialists. The remaining two patients underwent resection of NPs only because there was no mucopurulent discharge in sinuses when ESS was performed. NPs were resected from 16 patients. The number of eosinophils per high power field (HPF, 400×) was evaluated by a pathologist (A.M.), who was blinded to other participant characteristics. Patients were considered to have eosinophilic NPs if eosinophils were seen ≥ 70 /HPF in NPs(6). In this study, the number of eosinophils in sinus was also evaluated because some patients did not show NPs at ESS. Thus, eosinophilic CRS was defined if eosinophils were seen ≥ 70 /HPF in either NPs or sinus tissues.

Evaluation of asthma symptoms before ESS

At enrollment, the 36 CRS patients were considered as non-asthmatic by respiratory specialists (Y.K., M.T. and A.N.); they had never experienced typical asthma symptoms such as persistent wheezes, dyspnea and cough, and had not been given a diagnosis of asthma or anti-asthma treatment throughout their life. There were no audible wheezes on chest examination at study enrollment.

Evaluation of asthma symptoms after ESS

Typical asthma symptoms (dyspnea, wheezing, chest tightness, and cough, which were variable) and auscultation of wheezes were evaluated after ESS. Other conditions presenting with similar symptoms, such as heart failure and respiratory infection, were excluded using physical examination, blood tests, and chest X-rays. Chest X-rays were double-checked by a radiology specialist (Y.O.).

Statistical analysis

Statistical analysis was performed with JMP system version 11.2 (SAS Institute Japan, Tokyo, Japan). Values are expressed as medians (5th percentile, 95th percentile). Comparison of patients with the development of asthma symptoms group and those without asthma symptoms was performed using a Wilcoxon rank-sum test. Comparison of before and 12 months after ESS measurements were made using Wilcoxon single-rank test. For categorical variables, chi-square test or Fisher's exact test was applied. Cut-off values, sensitivity and specificity of significant factors associated with the development of asthma symptoms were estimated using a Receiver Operating characteristic (ROC) analysis. A p values of <0.05 was considered statistically significant when α error was set at 5%.

Results

Baseline characteristics, preoperative examinations, and pathological results of surgical specimens

The baseline patient characteristics, clinical indices, and ESS pathology results are presented in Table 1. Six patients ceased attending appointments at our hospital (Figure 1), thus 30 patients were followed up for 12 months after ESS, 7 (23%) of which complained of new asthma symptoms during this period. There were no significant differences in baseline characteristics between patients with and without the development of asthma symptoms after ESS. Indices of systemic and upper airway inflammation were also similar between the two groups (Table 1).

With respect to the lower airway indices, FeNO was significantly higher in patients with the development of asthma symptoms after ESS than in those without (Table 1, 50.7 ppb vs 22.4 ppb, $p = 0.02$). However, levels of sputum periostin, FEV₁, airway hyperresponsiveness, and recurrence rate of NPs did not differ between the two groups.

Although limited to a small number of patients, the number of eosinophils in NPs tended to be higher in the development of asthma symptoms group ($n = 3$) than in the non-asthma group ($n = 13$) (Table 1, 86/HPF vs 4/HPF, $p = 0.07$). When eosinophilic CRS was defined by the number of NP eosinophils being 70/HPF or more, as previously reported(6), its prevalence was higher in the development of asthma symptoms group than in the non-asthma group (Table 1, 100% vs 23%, $p = 0.04$). The prevalence of patients with eosinophils in sinus tissue ≥ 70 /HPF was similar between the two groups (Table 1). When performing a ROC analysis, FeNO levels of 36.4 ppb and eosinophils in NPs of 70/HPF showed high sensitivity and specificity (71% and 91% for FeNO, and 100% and 77% for eosinophils in NPs, respectively). However, we think the present sample size may not be tough enough to make the power analysis in statistics stable. Thus, these results are exploratory rather than confirmatory.

We also assessed biomarkers and characteristics between asthma symptom group and non-asthma group in the division into ECRS and non-ECRS. ECRS was defined if eosinophils counts were $\geq 70/\text{HPF}$ in NPs or sinus tissues. Five of 12 ECRS patients (42%) and two of 18 non-ECRS (11%) developed asthma symptoms within 12 months following ESS. ECRS patients tended to develop asthma symptoms within 12 months after ESS as compared to non-ECRS patients ($p = 0.08$). Among five ECRS patients who developed asthma symptoms, three had nasal polyps. Two of four CRSsNP patients showed eosinophil infiltration in sinus tissue ($\geq 70/\text{HPF}$). Therefore, 5 of 7 patients (71%) were considered to have ECRS. Although sample size may not be statistically enough, FeNO levels were numerically higher in asthma symptom group than in non-asthma group [50.7 ppb vs 30.6 ppb for ECRS group, and 51.2 ppb vs 21.7 ppb for non-ECRS group]. Other biomarkers such as blood eosinophils and sputum periostin were comparable between asthma symptoms and non-asthma groups.

Characteristics of patients with the development of asthma symptoms after ESS

The characteristics of patients who development asthma symptoms are presented in Table 2. Five patients had FeNO levels of ≥ 50.0 ppb at enrollment, and two of whom also had positive airway hyperresponsiveness test results. Medium doses of ICS (fluticasone propionate equivalent 400 μg a daily) were commenced immediately and we confirmed the improvement of symptoms thereafter. FeNO levels also declined in three of the patients after ICS treatment. One male patient showed the recurrence of NPs at 12 months following ESS. He had no lower respiratory symptoms such as cough, dyspnea and chest tightness at the time of enrollment but FeNO was 69.1 ppb. His resected NP had prominent infiltration of eosinophils (552/HPF). Nine months post ESS he complained cough, had increased levels of FeNO (108.0 ppb) and wheezes were audible on chest auscultation. A recurrence of NP was found by nasal endoscopy and sinus CT at 12 months after ESS.

All patients complained of new asthma-related symptoms such as nocturnal cough, dyspnea, and wheezing. Wheezes were audible in four patients on chest auscultation when complaining of asthma symptoms.

This suggests that they may have already had comorbid asthma without symptoms at enrollment (called “subclinical asthma”).

Changes in clinical indices by ESS intervention

The changes in clinical indices before and 12 months after ESS are presented in Table 3. In the non-asthma group, sputum periostin, sinusitis-related QoL, and radiological severity of CRS improved significantly with the intervention of ESS (Table 3). They also showed a trend toward of increased FEV₁ (Table 3). Forced vital capacity and maximum mid-expiratory flow were

significantly improved following ESS (Table 3). However, these significant or marginal improvements were not observed in the group who subsequently developed asthma symptoms.

Improvements in olfactory function and sinusitis-related QoL were marginally but not significantly greater in the non-asthma group as compared to the development of asthma symptoms group (Table 3). Otherwise, there was no difference in the changes of clinical indices between the two groups.

Discussion

CRS often coexists comorbid asthma(6–8, 26), though the exact pathophysiological determinant of the development of asthma symptoms remains unknown. Albeit exploratory findings, we have demonstrated for the first time to the best of our knowledge, that NPs eosinophilia ($\geq 70/\text{HPF}$) can be helpful for predicting the development of asthma symptoms within 12 months after ESS. An elevated level of preoperative FeNO also predicts the development of asthma symptoms in CRS patients who underwent ESS. Although further studies are necessary for confirmation, the present results suggest that allergy specialists should consider the evaluation of lower airway inflammation as well as upper airway in CRS patients, particularly CRSwNP (CRS with nasal polyp) patients, with no obvious history of asthma.

Both clinical and experimental studies have clearly demonstrated that sinus tissue inflammation is associated with lower airway inflammation(27, 28). Tissue eosinophilia in NPs is associated with the presence of asthma(6, 7) and recurrence of NPs (11). This indicates that eosinophilic NP is a strong inducer of type2 inflammation throughout the airways. Indeed, levels of FeNO and sputum periostin are higher in CRS patients than in healthy subjects irrespective of presence or absence of asthma(12, 29). Although this study demonstrates the pathological impact of NPs on the development of asthma symptoms after ESS in CRS patients, changes in asthma symptoms related to the reoccurrence of NPs remains unclear. We should evaluate the efficacy of targeting therapy for eosinophilic NPs on asthma in further studies.

One of the main messages of this study is the importance of FeNO measurements in clinical practice, not only for respirologists but also for otorhinolaryngologists. FeNO can be measured non-invasively, simply and repeatedly (23). High levels of FeNO are well-known as a feature of severe adult onset asthma, along with the presence of NPs (30). Clinicians should consider measurement of FeNO for CRS patients even if they do not have comorbid asthma. An increased awareness of the future risk of the development of asthma symptoms in CRS patients may lead to early treatment of asthma.

Our study has several limitations. Firstly, we restricted enrollment to patients due to undergo ESS because we were focusing on the role of histological characteristics of the sinus or NPs on the

lower airway inflammation and symptoms in CRS patients when establishing the study design. Also, other factors measured in the study may have been significantly different between the groups if the sample size was larger, such as serum periostin levels, absolute eosinophils counts, the number of eosinophils in NPs, or airway hyperresponsiveness. Therefore, the present results may be preliminary and exploratory rather than confirmatory. To determine whether eosinophils in NP and lower airway biomarkers including FeNO reflect the development of asthma symptoms in the near future, we should evaluate CRS patients who do not need ESS intervention. Despite these limitations, the present results argue the potential benefit of monitoring FeNO and evaluating eosinophils in NPs before performing ESS. Secondly, one may argue that we might have missed asthma diagnosis in patients with higher FeNO or having airway hyperresponsiveness prior to ESS. Some patients in non-asthma group may have comorbid subclinical asthma even if they were asymptomatic during the follow-up period. In the present study, symptoms and previous history of asthma were sufficiently scrutinized by asthma specialists before ESS. Additionally, evaluation of asthma when patients developed symptoms may have been insufficient. Indeed, we did not evaluate airway obstruction and its variability/reversibility when developing symptoms following ESS in the present study. We should have examined the changes in biomarkers at least soon after ESS. Evaluation of biomarkers soon after ESS may provide the additional information to predict the development of asthma symptoms in the near future. Meanwhile, as previously reported, FeNO is useful for diagnosis of asthma only when we evaluate it together with symptoms such as dyspnea, wheezes, and chest tightness(23, 31). Guidelines do not accept a diagnosis asthma based only the presence of either high levels of FeNO, airway hyperresponsiveness or airway reversibility, unless showing asthma-related symptoms. Furthermore, we confirmed the diagnosis of asthma by response to ICS. Even if patients already had subclinical asthma at enrollment, clinicians could utilize levels of FeNO and number of eosinophils in NPs as biomarkers that discriminate comorbid subclinical asthma among CRS patients. Third, this study involved just a 12-month observation period. This may not have been sufficient to capture all cases of CRS patients who would eventually develop asthma symptoms following ESS. This short follow-up may not be adequate to find biomarkers related to the development asthma symptoms after ESS. Nonetheless, we think higher levels of FeNO and the number of eosinophils in NPs before ESS would be useful information for otolaryngologists, respirologists, and allergists. Although our cohort is small, the present study shows the potential benefit of FeNO measurement and evaluating eosinophils in NPs before performing ESS. To confirm the findings of this study, longer term observation of the association between FeNO levels and the development of asthma symptoms in a longer cohort of CRS patients is necessary.

In conclusion, although an exploratory finding, the number of eosinophils in NPs can be an indicator of the risk of developing asthma symptoms within 12 months after ESS. A high preoperative level of FeNO is also a significant risk factor for the development of asthma symptoms. They may be a sign of comorbid subclinical asthma in CRS patients. Further studies of mechanisms

by which upper airway inflammation affects the development of type2inflammation in the lower airways are required.

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Authors' contributions

Y.K.: established the conception of the whole study, and contributed to the performance of diagnostic tests, the collection of data, the recruitment of patients, disease diagnosis and management, the acquisition and interpretation of data, and drafting the manuscript. K.F., R.K., and N.T. contributed to the performance of diagnostic tests, the collection of data, and the acquisition and interpretation of data. S.F., T.U., T.T., H.O., K.M., Y.I., and T.O. contributed to the diagnostic tests, the collection of data, and management of patients. J.O. and K.I. carried out the measurement of periostin. A.M. made specimens and assessed the infiltration of eosinophils in upper airway tissues. Y.O. contributed to assess the radiological severity of CRS. M.T. contributed to the recruitment of patients, disease diagnosis and management, and revision of the manuscript. M.S. and A.N. contributed to the recruitment of patients, disease diagnosis and management, interpretation of data, and revision of the manuscript.

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Conflict of interest

Y.K. reports research grants from Novartis Pharma, and Tanabe Mitsubishi pharma for the submitted work, and grants from MSD and Kyowa-Kirin corporations outside the submitted work. K.F. reports research grants from Novartis Pharma, and GSK outside the submitted work. S. F. reports personal fees from AstraZeneca, personal fees from Eli Lilly Japan outside the submitted work. H.O. reports research grant from Boehringer Ingelheim outside the submitted work. K.M. reports personal fees from Pfizer and Chugai Pharmaceutical outside the submitted work. T.O. reports personal fees from AstraZeneca, Eli Lilly Japan, Taiho Pharmaceutical, Pfizer, Chugai Pharmaceutical, MSD, Daiichi Sankyo, and Asahi Kasei Pharma, and research grants and personal fees from Kyowa Hakko Kirin, Boehringer Ingelheim, Ono Pharmaceutical, and Novaltis outside the submitted work. K.I. reports research grants from Shino-Test Corporation for the submitted work. M.T reports research grant from Pfizer outside the submitted work. M.S. reports research

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Figure 1. The recruitment flow of CRS patients without asthma in this study.

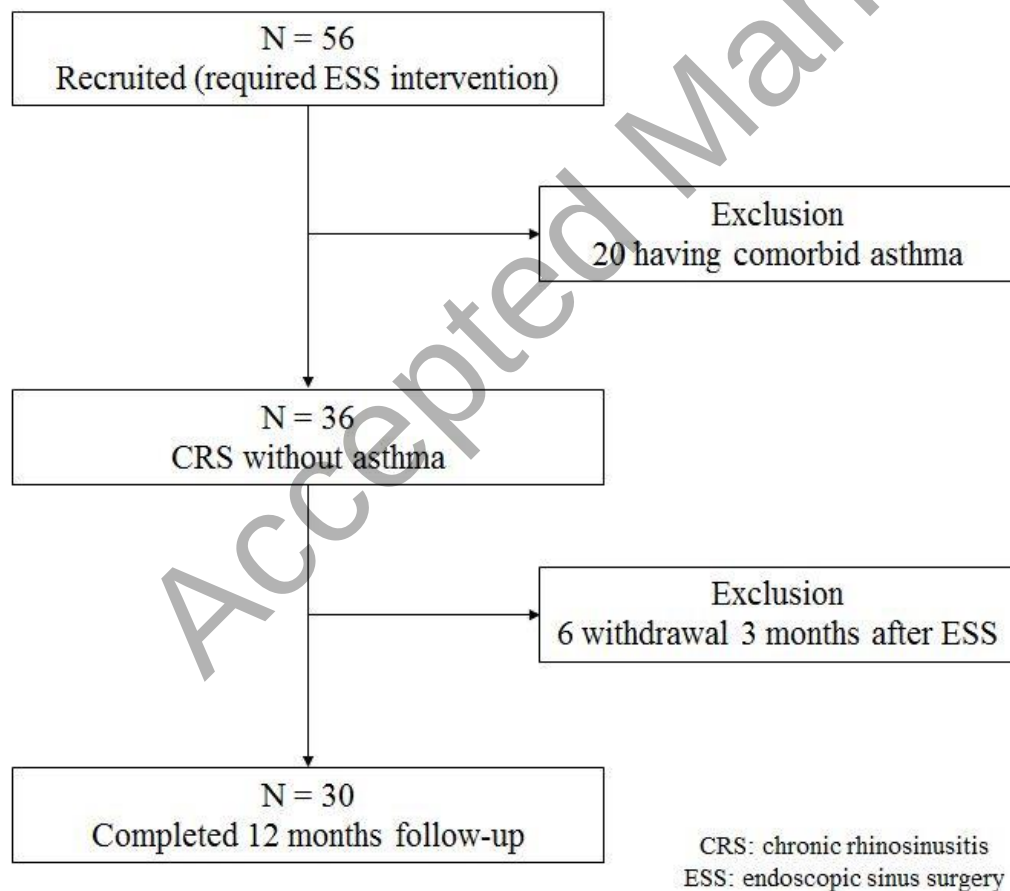


Table 1. Baseline patient characteristics, clinical indices, and pathological results of endoscopic sinus surgery.

	Entire cohort (n = 30)	The development of asthma symptoms (n = 7)	Non-asthma (n = 23)	p value
Age, years	63 (43, 78)	56 (41, 75)	64 (50, 77)	0.34
Sex, Males (%)	18 (60)	4 (57)	14 (61)	>0.99
Smoking status, never (%)	12 (40)	3 (43)	9 (39)	>0.99
Eosinophilic chronic rhinosinusitis, n (%)	12 (40)	5 (71)	7 (30)	0.08
Blood eosinophils, / μ L	154 (11, 416)	228 (88, 298)	140 (9, 487)	0.10
Serum total immunoglobulin E, IU/L	49.5 (9.5, 1128)	34.2 (9.4, 1146)	50.8 (14.3, 695)	0.48
Serum specific immunoglobulin E against <i>Staphylococcus aureus</i> enterotoxins. n (%)	4 (13)	1 (14)	3 (13)	>0.99
Atopic predisposition, n (%)	14 (46)	2 (29)	12 (52)	0.40
Aspirin/nonsteroidal anti-inflammatory drug sensitivity, n (%)	1 (3)	1 (14)	0 (0)	0.23
Serum periostin, ng/ml	79 (52, 122)	73 (44, 153)	79 (55, 119)	0.59
Sputum eosinophils, %	0.0 (0.0, 5.9)*	0.0 (0.0, 2.0)	0.0 (0.0, 7.5) [†]	0.64
Sputum periostin, ng/ml	9.4 (0.4, 37.8)*	12.0 (6.3, 25.9)	7.8 (0.4, 41.3) [†]	0.32
% Forced expiratory volume in one second predicted, %	98.7 (72.1, 122.9)	92.4 (84.5, 116.0)	99.4 (70.3, 124.5)	0.71
% Maximum mid-expiratory flow predicted, %	61.6 (29.6, 175.7)	61.5 (53.5, 175.7)	61.7 (29.6, 158.4)	0.38
Fractional nitric oxide, ppb	23.2 (8.5, 67.7)	50.7 (16.1, 98.7)	22.4 (7.2, 38.4)	0.02
Airway hyperresponsiveness, Dmin	50.0 (0.2, 50.0)	50.0 (0.1, 50.0)	50.0 (0.2, 50.0)	0.53
Lund-Mackay scores, points	9 (4, 19)	7 (4, 16)	9 (4, 20)	0.57
Olfactory score, points	6 (0, 10)	7 (1, 10)	6 (0, 9)	0.44
Sinonasal outcome test-22, points	21 (4, 61)	19 (9, 59)	22 (3, 59)	0.70
Surgical pathology				
Nasal polyps, presence (%)	16 (53)	3 (43)	13 (57)	0.67
Tissue eosinophils counts, nasal polyps, /HPF	10 (0, 350) [‡]	86 (72, 505) [§]	4 (0, 260)	0.07
Sinuses, / HPF	40 (0, 451) [¶]	101 (9, 159) [#]	31 (0, 487) ^{**}	0.54

Eosinophils in nasal polyps ≥ 70 / HPF, n (%)	6 (38) [‡]	3 (100) [§]	3 (23)	0.04
Median (5th percentile, 95th percentile), HPF: high power field (400 $\times$), *n = 27, †n = 20, ‡n = 16, §n = 3, n = 13, ¶n = 28, #n = 6, **n = 22. All patients were Japanese.				

Table 2. Characteristics and clinical indices of seven patients developing asthma symptoms.

Patient number	1	2	3	4	5	6	7
Age, years	56	64	67	78	37	50	53
Sex	Male	Male	Female	Male	Female	Female	Male
Smoking status, pack-year	5	15	0	3	0	0	60
Time until the development of asthma symptoms after ESS, months	12	9	6	12	12	7	7
FeNO before ESS, ppb	50.7	69.1	36.4	65.9	21.7	111.4	13.7
FeNO at the development of asthma symptoms, ppb	60.6	108.0	36.0	75.2	51.1	90.0	10.4
%FEV ₁ predicted before ESS, %	84.0	86.6	115.4	111.2	116.2	85.5	92.4
%FEV ₁ predicted at the development of asthma symptoms, %	75.2	91.4	106.6	108.5	106.7	83.3	90.1
Airway hyperresponsiveness at baseline	-	+	-	-	-	+	+
Blood eosinophils at baseline, / μ L	302	262	133	68	287	201	228
Serum periostin at baseline, ng/ml	93	41	56	179	84	71	73
Sputum eosinophils at baseline, %	0.8	2.0	0	2.0	0	0	0
Sputum periostin at baseline, ng/ml	14.2	27.2	7.1	10.1	12.0	22.8	6.0
Nasal polyps at ESS	-	+	-	-	+	+	-
Tissue eosinophils counts, nasal polyps, /HPF	N/A	552	N/A	N/A	86	70	N/A
Sinuses, /HPF	103	N/A	1	33	122	99	171
The presence of nasal polyps* within 12 months after ESS	-	+	-	-	-	-	-
Asthma-related symptoms before ESS	None	None	None	None	None	None	None
Developing asthma symptoms	Wheezing	Cough	Cough	Cough, dyspnea	Cough	Cough, dyspnea	Dyspnea, wheezing
Audible wheezes by auscultation before ESS	-	-	-	-	-	-	-
Audible wheezes by auscultation at the development of asthma symptoms	+	+	-	+	-	-	+

ESS: endoscopic sinus surgery, FeNO: fractional exhaled nitric oxide, FEV₁: forced expiratory volume in one second, N/A: not available, HPF: high power field (400×).

*Nasal polyps requiring surgical intervention, Airway hyperresponsiveness was considered positive if D_{min}, the cumulative dose of inhaled methacholine at the inflection point at which baseline respiratory resistance began to increase, represented ≤ 12.5 units.

Table 3. Clinical indices before and 12 months after endoscopic sinus surgery between patients with and without the development of asthma symptoms.

	The development of asthma symptoms group (n = 7)			Non-asthma group (n = 23)			P value*
	Before	12 months after	p value	Before	12 month after	p value	
Blood eosinophils, / μ L	228 (88, 298)	238 (37, 302)	0.84	140 (9, 487)	95 (6, 374)	0.61	0.60
Serum periostin, ng/ml	73 (44, 153)	71 (54, 99)	0.94	79 (55, 119)	82 (52, 115)	0.77	0.67
Sputum eosinophils, %	0.0 (0.0, 2.0)	2.5 (0.5, 7.1) [†]	0.13 [†]	0.0 (0.0, 7.5) [‡]	1.0 (0.0, 8.5) [§]	0.56 [§]	0.66
Sputum periostin, ng/ml	12.0 (6.3, 25.9)	3.8 (1.3, 27.5) [†]	0.44 [†]	7.8 (0.4, 41.3) [‡]	2.2 (0.6, 15.0) [§]	0.002 [§]	0.93
%FEV ₁ predicted, %	92.4 (84.5, 116.0)	96.4 (79.1, 108.0)	0.41	99.4 (70.3, 124.5)	101.9 (74.8, 124.6)	0.053	0.12
%MMF predicted, %	61.5 (53.5, 175.7)	74.9 (44.0, 189.2)	>0.99	61.7 (29.6, 158.4)	76.4 (21.4, 166.6)	0.004	0.45
Fractional nitric oxide, ppb	50.7 (16.1, 98.7)	51.1 (15.9, 72.3)	0.69	22.4 (7.2, 38.4)	22.2 (11.4, 41.3)	0.83	0.46
Lund-Mackay scores, points	7 (4, 16)	6 (1, 17)	0.30	9 (4, 20)	3 (0, 14)	<0.0001	0.20
Olfactory score, points	7 (1, 10)	6 (1, 10)	>0.99	6 (0, 9)	7 (0, 9)	0.08	0.09
SNOT-22, points	19 (9, 59)	22 (11, 50)	>0.99	22 (3, 59)	6 (2, 24)	0.002	0.07
Recurrent nasal polyps, presence (%) [¶]	-	1 (33) [#]	-	-	3 (23) ^{**}	-	>0.99

Median (5th percentile, 95th percentile), *Comparison of the differences in clinical valuables between the patients with asthma and without asthma onset.

FEV₁: forced expiratory volume in one second, MMF: maximum mid-expiratory flow.

[†] $n = 5,$

[‡] $n = 20,$

[§] $n = 17,$

^{||} $n = 22,$

[¶] $n = 16,$

[#] $n = 3,$

^{**} $n = 13.$