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Allergology International 68 (2019) 225–232

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Original Article

# Reduced nasal nitric oxide levels in patients with eosinophilic chronic rhinosinusitis

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ARTICLE INFO

*Article history:*
Received 23 April 2018
Received in revised form
30 August 2018
Accepted 14 September 2018
Available online 19 October 2018

*Keywords:*
Asthma
Chronic rhinosinusitis
Eosinophilic chronic rhinosinusitis
Eosinophilic inflammation
Nasal nitric oxide level

*Abbreviations:*
ECRS, eosinophilic chronic rhinosinusitis; CRSwNP, chronic rhinosinusitis with nasal polyps; FeNO, fraction of exhaled nitric oxide; NO, nitric oxide; CRS, chronic rhinosinusitis; non-ECRS, non-eosinophilic chronic rhinosinusitis; ESS, endoscopic sinus surgery; CT, computed tomography; SEM, standard error of the mean; ROC, receiver operating characteristic; AUC, area under the curve; t-PA, tissue plasminogen activator; LRP-1, low-density-lipoprotein receptor-related protein-1

ABSTRACT

*Background:* In Eosinophilic chronic rhinosinusitis (ECRS), it is difficult to estimate the refractoriness and recurrence risk for each patient. Fraction of exhaled nitric oxide (FeNO) is known as a biomarker of eosinophilic inflammation in lower airway. It has been reported that nasal NO has some crucial functions in the upper and lower airways. However, in upper airway, paranasal sinuses, the usefulness of NO measurement remains controversial. The purpose of this study is to identify the usefulness of nasal NO measurement in ECRS and the involvement of nasal NO in the pathogenesis of ECRS.

*Methods:* We compared the nasal NO levels of ECRS, non-ECRS, and normal control groups. Correlation between nasal NO levels and clinical findings were observed. Then, we compared nasal NO levels before and after endoscopic sinus surgery (ESS). We also examine whether nasal NO levels might discriminate ECRS by the receiver operating characteristic (ROC) curve analysis.

*Results:* Nasal NO levels were significantly decreased in ECRS compared to the other two groups. Moreover, nasal NO levels in ECRS significantly and negatively correlated with eosinophil levels and CT score. However, they did not correlate with the nasal polyp score. Nasal NO levels were not upregulated soon after opening the sinus ostium by ESS. The ROC curves for nasal NO levels were used to discriminate all CRS patients and ECRS patients from normal controls.

*Conclusions:* Nasal NO may be useful as a marker of ECRS severity and low nasal NO levels in ECRS may contribute to its pathogenesis.



## Introduction

Eosinophilic chronic rhinosinusitis (ECRS) is a refractory and intractable disease that significantly impairs quality of life. Characterized by chronic inflammation of paranasal sinuses and associated with infiltration of activated eosinophils in nasal polyp tissue, it is considered a type of chronic rhinosinusitis with nasal polyps (CRSwNP).[1] In Japan, histologically ECRS was defined as an average count of more than 70 eosinophils per microscopic field (400×) in three submucosal fields of nasal polyp tissue.[2] Patients with ECRS have long-term nasal obstruction, loss of smell, viscous mucus production, and post nasal drip. There is a strong tendency for nasal polyps to recur despite medical and surgical interventions,[3–5] which is confounded by the fact that the clinical course is variable, making it difficult to estimate the refractoriness and recurrence risk for each patient. ECRS is, therefore, a particularly problematic disease for both the patient and otorhinolaryngologist. However, if an easy and objective marker was available for assessing the severity of rhinosinusitis, it would be invaluable in treating the disease.

ECRS is often associated with lower airway disease, with asthma being a known risk factor for refractory disease.[1] In asthmatic patients, the fraction of exhaled nitric oxide (FeNO) has been shown to

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Peer review under responsibility of Japanese Society of Allergology.

https://doi.org/10.1016/j.alit.2018.09.005

increase after allergic asthmatic reactions and to decrease after inhaled corticosteroid use.[6] In this way, FeNO measurement is a non-invasive and useful method for evaluating eosinophilic airway inflammation that is used for the diagnosis and management of asthma.[7–9]

The upper airways, especially the paranasal sinuses, are the major source of NO in the respiratory tract.[10] Several studies have suggested that nasal NO plays several physiological roles. For example, NO contributes to local host defenses against bacterial, viral, and fungal infection, and helps to maintain a bacteriostatic state in the paranasal sinus. Indeed, low nasal NO levels in patients with cystic fibrosis and primary ciliary dyskinesia tend to increase the susceptibility of these patient groups to airway infections.[11,12] Moreover, NO regulates ciliary motility, and low NO levels impair muco-ciliary function in the upper airways.[13] It has been reported that nasal NO derived from the upper airway may have pivotal protective functions in the lower airway at sites of inflammation. In addition, nasal NO was shown to increase arterial oxygen tension and to decrease pulmonary vascular resistance. As a result, nasal NO has a role in modulating cardiopulmonary function in humans.[14]

Nasal NO levels can be measured noninvasively and easily in clinical practice, and they might be clinically useful as a marker for assessing the severity of ECRS. In the current study, we therefore investigated the nasal NO levels in patients with chronic rhinosinusitis (CRS) by the presence or absence of eosinophilia (i.e., ECRS and non-ECRS groups, respectively). We also assessed the correlation between nasal NO levels and clinical manifestations.

## Methods

### *Patients*

This was a prospective case-control study in which we compared 25 patients with ECRS, 45 patients with non-ECRS, and 33 normal volunteers served as controls. All patients with CRS were recruited from the Department of Otorhinolaryngology Head & Neck Surgery, University of Fukui, and were undergoing nasal surgery—specifically, endoscopic sinus surgery (ESS)—for the first time. Surgical procedures for ESS were classified into 5 types as proposed by Japanese Rhinologic Society in 2013.[15] Basically, we have performed ESS type IV, the pansinus procedure, with the aim to enlarge and maintain the patency of each sinus ostia on affected side. In 45 non-ECRS patients, 25 underwent ESS type IV on bilateral sides, 8 out of 25 underwent septoplasty at the same time and 1 out of 25 underwent both septoplasty and inferior turbinectomy at the same time. On the other hand, 20 underwent ESS type IV on one side, 8 out of 20 underwent septoplasty at the same time and 1 out of 20 underwent both septoplasty and inferior turbinectomy at the same time. In 25 ECRS patients, 23 patients underwent ESS type IV on bilateral sides and 2 patients needed ESS type V, the extended procedure beyond the sinus wall. 12 out of 25 ECRS patients underwent septoplasty at the same time and 2 out of 25 underwent both septoplasty and inferior turbinectomy at the same time.

The patients had not received local or systemic steroids for at least 4 weeks before surgery. After ESS, we have used systemic steroid (betamethasone) for about two weeks, local steroid by the nasal spray for about a month, macroride antibiotics and carbocisteine for about two weeks in non-ECRS patients. On the other hand, we have used systemic steroid (betamethasone) for about a month, local steroid by the nasal spray for about a month, macroride antibiotics and carbocisteine for about two weeks, and LT antagonists (montelukast) for long-term in ECRS patients after ESS. However, the administration period is varied according to each condition. All provided informed consent. The protocol and consent forms were approved by the ethics committee of the University of Fukui and were consistent with the ethical principles of the Declaration of Helsinki.

Histologically ECRS was defined as an average count of more than 70 eosinophils per microscopic field (400×) in three submucosal fields of the ethmoidal cavity or nasal polyp tissue.[2]

Each patient computed tomography (CT) scan of paranasal sinuses was graded based on Lund Mackay CT scoring system.[16] This score is 0 = no opacification; 1 = partial opacification; and 2 = complete opacification, while the ostiomeatal complex score is 0 = not occluded or 2 = occluded. Each sinus is staged and scored separately. The sinus scores were summed, and the combined score was scaled up to range from 0 to 24, with higher scores indicating a worse status.[17]

The nasal endoscopic polyp scores were staged according to polyp size, as follows: 0 = no polyps; 1 = small polyps in the middle meatus, but not reaching below the inferior border of the middle turbinate; 2 = polyps reaching below the lower border of the middle turbinate; 3 = large polyps reaching the lower border of the inferior turbinate, or polyps medial to the middle turbinate; and 4 = large polyps causing complete obstruction of the inferior nasal cavity. The total polyp scores were calculated bilaterally (ranging from 0 to 8), with higher scores indicating a worse status.[18,19]

Blood samples were taken to perform complete blood counts and measure eosinophils. When taken, nasal tissues were immediately fixed in 10% formalin, embedded in paraffin, and cut into thin sections before being stained with hematoxylin and eosin. The number of eosinophils in the nasal tissues was counted in a high-powered field (×400) in three submucosal fields of ethmoidal cavity or nasal polyp tissue, and the mean of three values was calculated.[2]

### *Measurements of nasal NO*

Oral and nasal FeNO levels were measured using a Sievers Nitric Oxide Analyzer (NOA 280i; GE Analytical Instrument) based on American Thoracic Society/European Respiratory Society guidelines.[20] In oral FeNO measurements, patients were instructed to exhale at a flow rate of 50 mL/s through a disposable mouthpiece. On the other hand, in nasal FeNO measurements, patients were advised to exhale at a flow rate of 50 mL/s through a nasal olive placed in the nostril under visual control on a computer screen. Measurements were performed three times at least, and the mean of three values was calculated for analysis. The nasal NO level used for analysis was determined by subtracting the oral FeNO level from the nasal FeNO level.[21] To evaluate the effect of ESS on nasal NO levels in patients, we measured nasal NO levels preoperatively and at 3 and 6 months postoperatively.

### *Measurements of nasal airflow resistance*

Nasal airflow resistance was measured using a rhinomanometer (RHINORHEOGRAPH MPR-3100, NIHON KOHDEN) by the anterior method. The measurements were performed one by one side. Nasal airflow resistance was determined by the calculated value at an inspiratory reference pressure of $\Delta P$ 100 Pa and expressed as $Pa/cm^3/sec$ according to the Japanese Guidelines for Rhinomanometry proposed by Japan Rhinologic Society. Total nasal airflow resistance was calculated by Ohm's law.[22,23]

### *Statistical analysis*

All data are reported as means ± SEM unless otherwise noted. Differences between groups ware analyzed with the Kruskal–Wallis ANOVA with Dunnett *post-hoc* testing and the Mann–Whitney *U*-test. Repeated measured data was analyzed with the Friedman test with *post-hoc* testing. Correlations were assessed by using

Spearman rank correlation. We drew a ROC curve to calculate the AUC to discriminate ECRS patients from normal subjects. The optimal cut-off value was determined by the Youden index based on the ROC. A $P$ value of less than 0.05 was considered to indicate statistical significance.

## Results

### *Subject characteristics*

In this prospective study, we compared 25 patients with ECRS, 45 patients with non-ECRS, and 33 normal controls. Their clinical characteristics are shown in Table 1. The following clinical manifestations were significantly higher in the ECRS than in the non-ECRS group: comorbid bronchial asthma ($P < 0.001$), blood eosinophil percentage ($P < 0.001$), eosinophil count in nasal polyp tissue ($P < 0.001$), CT score ($P < 0.01$), and nasal polyp score ($P < 0.001$). Smoking history and comorbidity of allergic rhinitis were no significant difference between ECRS and non-ECRS group. In this study, the diagnosis of allergic rhinitis was based on the presence of nasal symptoms and positive allergen-specific IgE antibody against cedar pollens and house dust mites, the major allergen contributing to allergic rhinitis in Japan.

### *Nasal NO levels in patients with CRS*

To measure NO production levels in paranasal sinuses, we have determined the nasal NO level according to subtract the oral FeNO level from the nasal FeNO level (Fig. 1A). Nasal NO levels were significantly lower in the CRS group (both ECRS and non-ECRS) than in the control group. In addition, nasal NO levels were significantly lower in the ECRS group ($P < 0.001$) compared to the other two groups. On the other hand, there was no significant difference in nasal FeNO levels among the three groups. Oral FeNO levels in ECRS were significantly higher compared to the other two groups ($P < 0.01$).

To exclude the possibility that FeNO levels in lower airway (oral FeNO levels) affect nasal NO levels, we examined the correlation between nasal NO levels and oral FeNO levels (Fig. 1B). No significant correlation was found between the paired parameters, suggesting that nasal NO levels were independent of oral FeNO levels.

In both ECRS and non-ECRS group, with or without asthma, the significant difference of nasal NO levels was not found. Similarly, there was no significant difference in nasal NO levels between the patients with or without allergic rhinitis in both ECRS and non-ECRS group. In addition, with or without smoking history, there was no significant difference in nasal NO levels between the patients in both ECRS and non-ECRS group (Fig. 1C).

**Table 1**
Subject characteristics.

| | Normal control | Non-ECRS | ECRS | |
|---|---|---|---|---|
| Number | 33 | 45 | 25 | |
| Age | 37.7 ± 1.7 (25–53) | 50.2 ± 2.6 (18–76) | 54.8 ± 2.9 (32–77) | *** |
| Smoking | – | 14 | 3 | ns |
| Asthma | 0 | 3 | 11 | *** |
| Allergic rhinitis | – | 22 | 11 | ns |
| Blood eosinophils (%) | – | 3.3 ± 0.3 (0.5–12.3) | 7.7 ± 0.8 (2.1–17.8) | *** |
| Tissue eosinophils (cells/HPF) | – | 8.1 ± 2.1 (0–66) | 137.5 ± 16.8 (70–299) | *** |
| CT score | – | 8.6 ± 0.8 (1–24) | 13.0 ± 1.3 (2–23) | ** |
| Nasal polyp score | 0 | 2.3 ± 0.4 (0–7) | 4.2 ± 0.3 (2–8) | *** |

Data are shown as mean with ranges in parenthesis.
**$p < 0.01$; ***$p < 0.001$: significant difference compared with the other group.

### *Correlation between nasal NO levels and clinical findings in patients with CRS*

Recently, we demonstrated that elevations in the blood eosinophil percent and eosinophil count in nasal polyp tissue were significantly associated with nasal polyp recurrence in CRS.[2] We therefore examined whether nasal NO levels correlated with eosinophil levels in the blood or nasal polyp tissue. Nasal NO levels were significantly and negatively correlated with both the percentage of blood eosinophils ($r = -0.2434$, $P < 0.05$) and the number of eosinophils in nasal polyp tissue ($r = -0.3652$, $P < 0.001$) (Fig. 2A,B). Thus, eosinophilic inflammation appeared either to downregulate NO synthesis or to inhibit NO diffusion in the nasal cavity and paranasal sinuses.

Next, we evaluated the correlation between nasal NO levels and CT scores (Fig. 3A) and showed that the nasal NO levels significantly and negatively correlated with CT score of patients with CRS ($r = -0.4303$, $P < 0.001$). Particularly, when participants were analyzed in non-ECRS and ECRS groups, the correlation between nasal NO level and CT score was stronger in the ECRS group than in the non-ECRS group (Fig. 3B,C). These results are consistent with nasal NO downregulation being responsible not only for the severity of paranasal inflammation but also for the eosinophilic inflammation of the paranasal sinuses.

To exclude the possibility that obstruction of the middle nasal meatus contributed to reducing NO levels, we investigated the correlation between nasal NO levels and nasal airflow resistance. The result showed that nasal NO levels in patients with CRS did not correlate with the nasal airflow resistance (Fig. 4A,B). In addition, we investigated the correlation between nasal NO levels and the nasal polyp score. Nasal NO levels in patients with CRS did not correlate with the nasal polyp score. Equivalent results were obtained in both ECRS and non-ECRS group (Fig. 4C,D,E), suggesting that the low nasal NO levels observed in CRS were not simply a consequence of middle nasal meatus obstruction by nasal polyps.

### *Changes in nasal NO levels before and after ESS*

Figure 5 shows the change in nasal NO levels from before to 6 months after surgery. We have examined the patients after surgery at least once a month, so the post-operative treatments have been controlled. In 30 non-ECRS patients, 7 out of 30 had used systemic and/or local steroid for more than 3 months after ESS and 4 out of 7 had used systemic and/or local steroid for more than half year after ESS. On the other hand, in 22 ECRS patients, 6 out of 22 had used systemic and/or local steroid for more than 3 months after ESS and 5 out of 6 had used systemic and/or local steroid for more than half year after ESS. The mean nasal NO levels in 30 patients with non-ECRS was 60.8 ± 5.2 parts per billion (ppb) preoperatively, 66.7 ± 6.8 ppb at 3 months, and 68.1 ± 6.0 ppb at 6 months after surgery (Fig. 5A). The mean nasal NO levels in 22 patients with ECRS was 42.3 ± 6.9 ppb before operation, 44.8 ± 5.6 ppb at 3 months, and 50.8 ± 7.4 ppb at 6 months after nasal surgery (Fig. 5B). There was no significant difference in nasal NO levels after ESS between bilateral and one side. There was no significant difference in nasal NO levels after ESS between type IV and type V and there was no significant difference in nasal NO levels after ESS with or without septoplasty and inferior turbinectomy. In addition, though there was a tendency to slightly decrease nasal NO levels under steroid use, there was no significant difference in nasal NO levels between two groups after ESS. Despite there being no statistically significant difference in the nasal NO levels among the periods, there was a trend for levels to increase gradually after surgery.

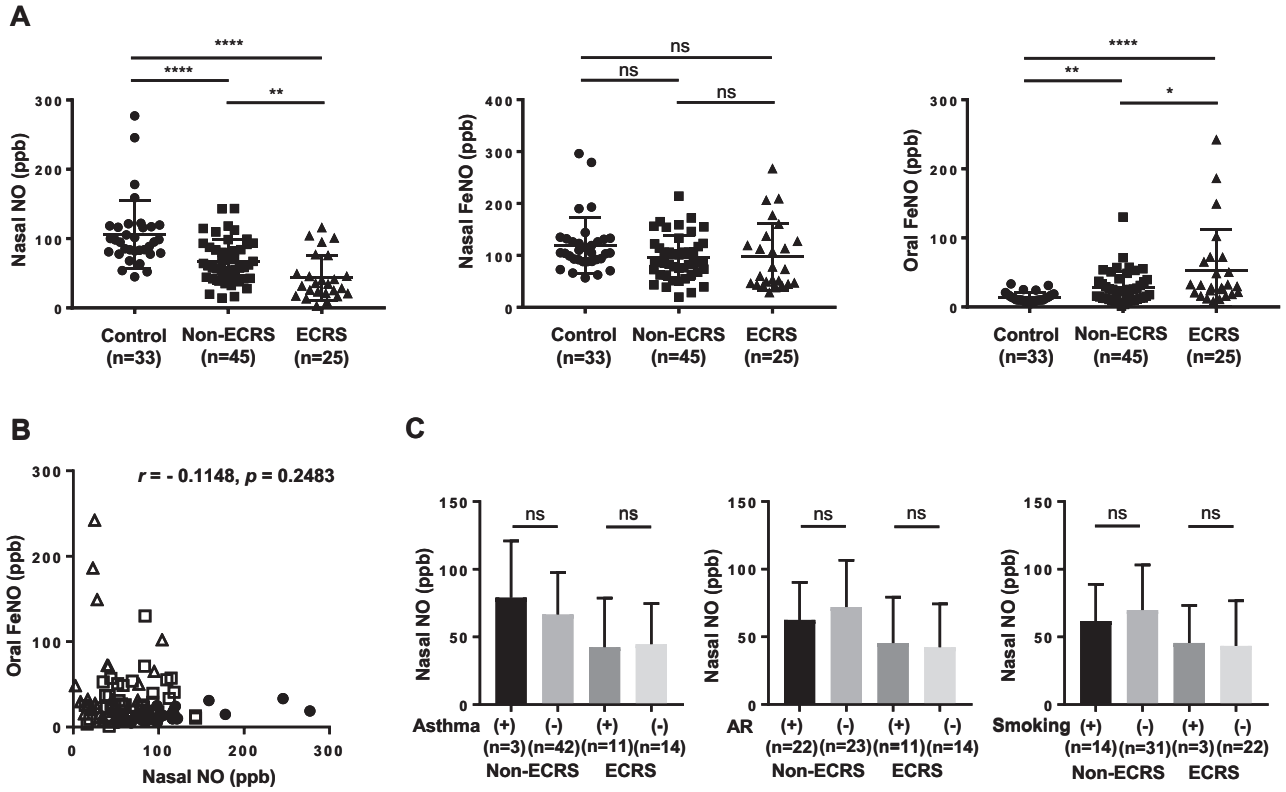


**Fig. 1.** Nasal NO levels in the patients with CRS. **(A)** The nasal NO levels, nasal FeNO levels, and oral FeNO levels (ppb) in normal control subjects (n = 33), non-ECRS patients (n = 45), and ECRS patients (n = 25). **(B)** Correlation between nasal NO levels (ppb) and oral FeNO levels (ppb). △ECRS □Non-ECRS ●Control. **(C)** The comparison of the nasal NO levels (ppb) with or without the comorbidity, asthma and allergic rhinitis, and smoking history. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{****}p < 0.0001$.

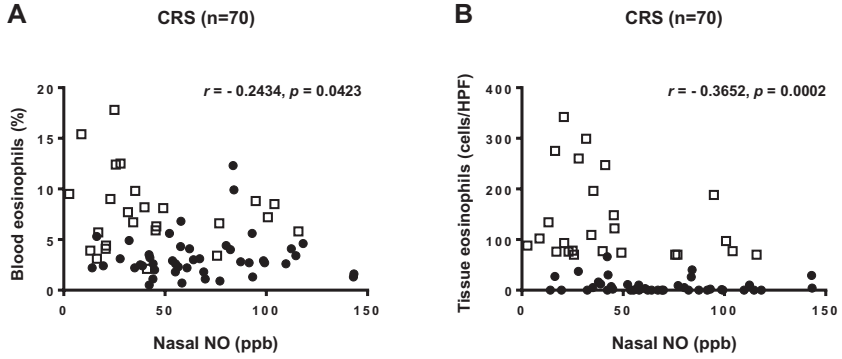


**Fig. 2.** The inverse correlation was shown between nasal NO levels and blood eosinophils and tissue eosinophils in the patients of CRS. Correlation between nasal NO levels (ppb) and **(A)** blood eosinophils percentage and **(B)** number of tissue eosinophils (cells/HPF) in the patients of CRS (n = 70). ●Non-ECRS □ECRS.

*Correlation of nasal NO level with the JESREC score*

We recently established the JESREC score as a tool for diagnosing and classifying ECRS without the need for biopsy specimens, based on assessment of bilateral disease sites, nasal polyps, CT findings, and peripheral eosinophilia. The presence of bilateral disease sites is 3 points; the presence of nasal polyps is 2 points; ethmoid ≧ maxillary for CT shadow dominant is 2 points; 2< ≦5% for peripheral blood eosinophil is 4 points; 5< ≦10% for peripheral blood eosinophil is 8 points; and 10% < for peripheral blood eosinophil is 10 points. A score higher than 11 points indicates ECRS. Additionally, CRS can be classified into four groups according to blood eosinophilia, ethmoid-dominant shadow on CT, and the presence of comorbidity (bronchial asthma, aspirin intolerance, and non-steroidal anti-intolerance), which are significantly correlated with the rate of recurrence and refractoriness.[2] We assessed the relationship between nasal NO levels and the JESREC score. Although there was no significant correlation between nasal NO levels and the JESRSC score in ECRS and non-ECRS group, there was a significant and negative correlation between nasal NO levels and the JESREC score in whole patients with CRS (Fig. 6). Nasal NO levels could be a suitable biological marker for assessing the recurrence rate and the refractoriness of CRS.

*Receiver operating characteristic (ROC) curve analysis*

Figure 7 shows that the ROC curves for nasal NO levels were used to discriminate all CRS patients and ECRS patients from

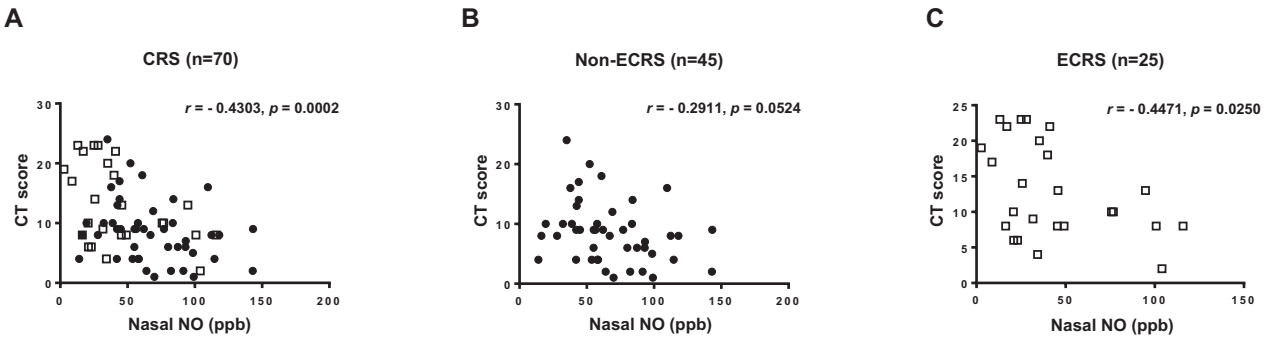


**Fig. 3.** The inverse correlation was shown between nasal NO levels and CT score in the patients of CRS. Correlation between nasal NO levels (ppb) and CT score **(A)** in the patients of CRS (n = 70), **(B)** in non-ECRS patients (n = 45), and **(C)** in ECRS patients (n = 25).

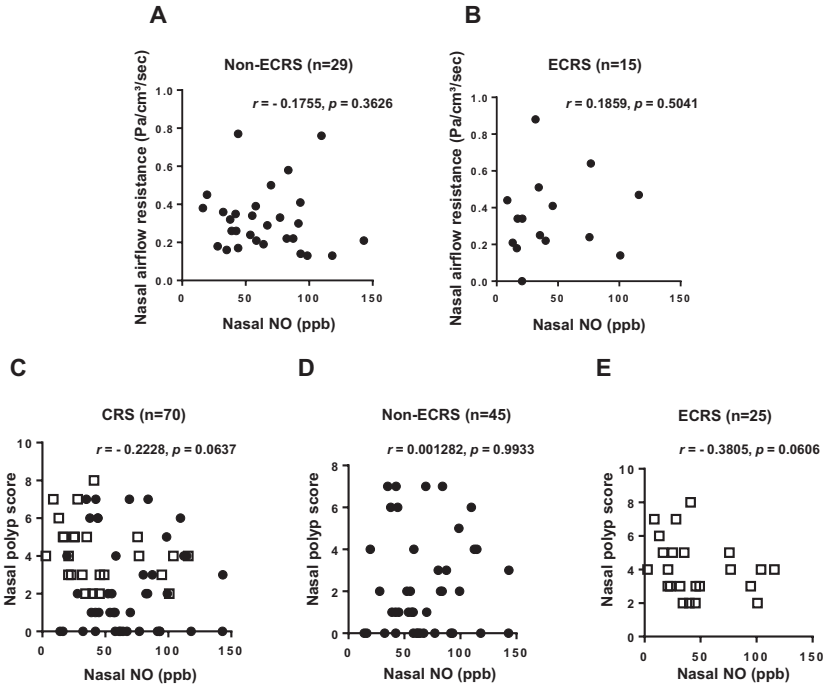


**Fig. 4.** Nasal airflow resistance and nasal polyp score were uncorrelated to nasal NO levels in the patients of CRS. Correlation between nasal NO levels (ppb) and nasal airflow resistance (Pa/cm$^3$/sec) **(A)** in non-ECRS patients (n = 29) and **(B)** in ECRS patients (n = 15). Correlation between nasal NO levels (ppb) and nasal polyp score **(C)** in the patients of CRS (n = 70), **(D)** in non-ECRS patients (n = 45), and **(E)** in ECRS patients (n = 25).

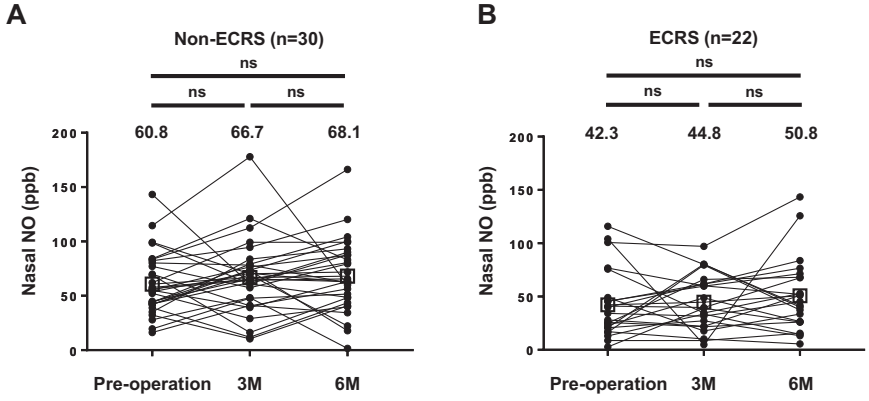


**Fig. 5.** There were no significant time-course changes in the patients of CRS. Time-course changes in nasal NO levels (ppb) in **(A)** non-ECRS patients (n = 30) and **(B)** ECRS patients (n = 22) pre-operation, at 3 months (3M), and 6 months (6M) after ESS. Time-dependent changes in each patient are shown by connecting the individual points. Hollow squares and numbers listed in the above indicate mean values.

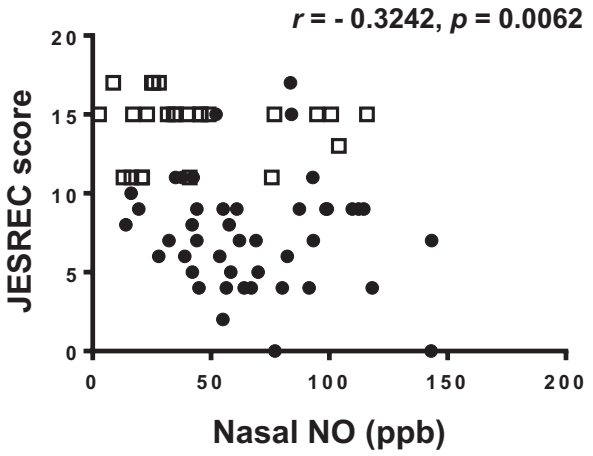


**Fig. 6.** The inverse correlation was shown between nasal NO levels and JESREC score in the patients of CRS. Correlation between nasal NO levels (ppb) and JESREC score in the patients of CRS (n = 70). ●Non-ECRS □ECRS.

normal controls. The optimal cut-off points of nasal NO levels were 77.3 ppb (with 71.4% sensitivity and 84.9% specificity) to differentiate the all CRS patients and 53.0 ppb (with 76.0% sensitivity and 97.0% specificity) to differentiate the ECRS patients. These results clearly indicate the superiority of measurement of nasal NO levels.

## Discussion

In the current study, we demonstrated that nasal NO levels in patients with CRS were significantly lower than in healthy controls. In addition, nasal NO levels were significantly lower in the ECRS group compared to the other two groups regardless of the comorbidity, such as allergic rhinitis and asthma, and smoking history. Furthermore, we found no significant correlation between nasal NO levels and oral FeNO levels, suggesting that the low nasal NO levels observed in patients with CRS were not only the because of the high oral FeNO levels (Fig. 1).

Several studies have indicated that the mucous membranes of the paranasal sinuses are a major source of NO in the respiratory tract. These studies have also shown that nasal NO plays a pivotal role in protecting and maintaining homeostasis of the whole airway, including the lung, through its anti-inflammatory effects.[11,12,14] It has been posited that nasal NO could modulate lung function and improve ventilation–perfusion matching, with lower NO levels being related to poorer lung function in both adults and children.[24] However, the molecular mechanisms underlying impaired NO production in the paranasal sinuses are still largely unknown.

Patients with CRS, especially those with ECRS, typically have extensive mucosal dysregulation and chronic inflammation. Therefore, a possible mechanism is that mucosal damage in the paranasal sinuses causes reduced nasal NO production that leads to CRS. Consistent with this theory, and in line with a previous report,[25] we found a significant and negative correlation between the nasal NO levels and the CT score (Fig. 3). Thus, nasal NO levels might reflect disordered mucosa in the paranasal sinuses of patients with CRS. Another possibility is that obstruction of the middle nasal meatus contributes to reduced nasal NO levels. However, we showed that there was no correlation between nasal NO levels and the nasal polyp score, and the nasal airflow resistance (Fig. 4). In addition, we showed that nasal NO levels were not upregulated soon after opening the sinus ostium by ESS (Fig. 5). These results indicated that the low nasal NO levels observed in patients with CRS were not simply the consequence of middle nasal meatus obstruction by a nasal polyp or morphological abnormality but the consequence of production decrease of nasal NO in the paranasal sinuses. Usually after surgery mucosa in paranasal sinuses is reepithelialization completely within 6 months at least and comes to look normal condition. However, we speculated that full recovery of NO production process required a little more time. In fact, in this study, although not statistically significant, nasal NO levels in patients with CRS showed an increasing trend after surgery. According to these results, NO production in paranasal sinuses were downregulated by mucosal damage especially in eosinophilic inflammation and rather than nasal NO levels being upregulated soon after opening the sinus ostium, they might instead only be upregulated as the paranasal sinus mucosa recovers its integrity. But further study is required.

Recently, we demonstrated that fibrin accumulates excessively in the nasal polyp tissue of patients who have CRSwNP. We also showed that this is involved in a reduction of tissue plasminogen activator (t-PA) levels, which is associated with converting plasminogen to plasmin during fibrinolysis. We have also reported that t-PA is expressed constitutively in nasal epithelial cells and that t-PA expression is significantly downregulated by the stimulation with the STAT-6-activating Th2 cytokines IL-4 or IL-13.[26] Growing evidence suggests that t-PA can play as a cytokine and bind to cell membrane receptor low-density-lipoprotein receptor-related protein-1 (LRP-1). Independent of its proteolytic activity, when t-PA binds to LRP-1 it induces receptor tyrosine phosphorylation and triggers intracellular signal transduction, NF-k-B signaling pathway, that induces NO production through the expression of inducible

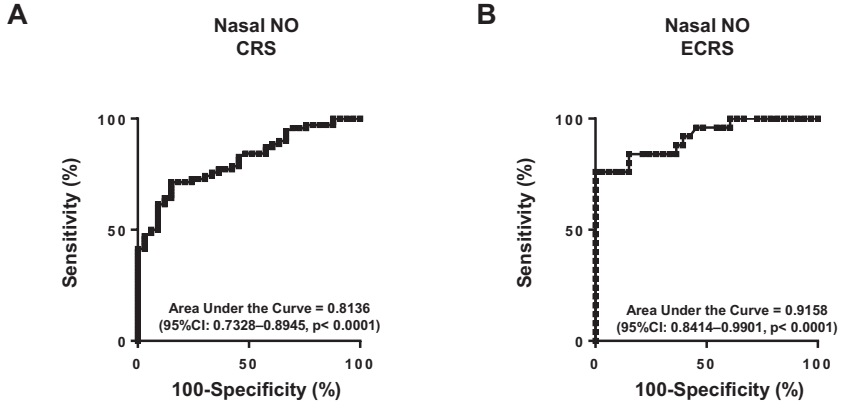


**Fig. 7.** ROC curve analysis for predictive values of nasal NO levels. The ROC curve and the area under the ROC curve (AUC) values in **(A)** all CRS and **(B)** ECRS patients.

nitric oxide synthase (iNOS) in human central nervous system. In human central nervous system, LRP-1 expression has been seen in perivascular astrocyte, vascular smooth muscle cells, macrophages, and neutrophils.[27,28] In addition, in this study, we detected LRP-1 expression in nasal mucosa by immunohistochemistry and real-time PCR, and LRP-1 expression in ECRS was downregulated compared to non-ECRS (data not shown). Given that the nasal mucosa in ECRS has a high tissue eosinophil count, demonstrating a skew toward Th2 cytokine expression, a Th2-polarized inflammatory milieu might be involved in reducing nasal NO production in ECRS through downregulated t-PA expression. However, it is in contradiction with some reports that iNOS expression is increased in ECRS.[29,30] It requires further study to reveal the mechanism of reduced NO production in CRS.

During wound healing, fibrin matrix deposition is replaced with collagen produced by fibroblasts.[31] NO is important to wound healing based on evidence that it induces collagen expression in human nasal polyp-derived fibroblasts.[32] Reduced NO levels might therefore cause collagen production to be downregulated, thereby inhibiting fibrin removal and wound healing that may prolong inflammation and edema in the nasal mucosa. This, in turn, can result in nasal polyp development, which is a hallmark of ECRS. Consistent with this, nasal polyp tissue is characterized histologically by extensive edema and reduced collagen.[33] Therefore, low nasal NO levels in CRS, especially in ECRS, might be responsible for the formation of intractable nasal polyps.

We previously reported, in the epithelium of nasal polyps in patients with CRS, there was a profound increase in the mast cell count and in mast cell activation.[34] Activated mast cells can release various preformed mediators and *de novo* synthesized proinflammatory mediators that might contribute to nasal polyp development. Histamine is one of such mediators that can facilitate vasodilatation and vascular permeability, resulting in tissue edema. Chemokines derived from mast cells may play a role in the recruitment of eosinophils and other cells found in nasal polyps.[35] Staphylococcal colonization is also common in the nasal polyps of patients with CRS, and it is possible that staphylococcal enterotoxin acts as an allergen, together with aeroallergens, to induce mast cell activation.[36,37] Because NO contributes to the local host defense against bacterial infection and because it regulates ciliary motility for adequate clearance of foreign material from the respiratory tract,[14] decreased nasal NO production might be involved in the pathogenesis of CRS.

Previous studies have demonstrated that FeNO is an important biomarker of eosinophilic inflammation in lower airway diseases like asthma and non-asthmatic eosinophilic bronchitis.[38] In asthma, the FeNO level is significantly and positively correlated with the number of eosinophils in bronchoalveolar lavage fluid, induced sputum, and airway mucosal tissue.[39–41] We therefore examined the correlation of nasal NO levels with the eosinophil count in nasal polyp tissue and peripheral blood samples in patients with CRS. This investigation revealed that there was a significantly negative correlation between nasal NO levels and eosinophils levels in both the nasal polyp tissue and peripheral blood of patients with CRS (Fig. 2). However, we speculate that there is a significant difference between the mucosa in the paranasal sinuses and the mucosa in the lower airways in terms of the response to eosinophilic inflammation, such as the mechanisms of NO production. For example, polyps or edema of mucosa have been formed in paranasal sinus mucosa as reaction to eosinophilic inflammation, but polyps or mucosal edema have never been formed in lower respiratory tract mucosa in asthma. In addition, a previous report suggested that there was a heterogeneous responsiveness of fibroblast populations to TGF-$\beta_1$ in the airways and that this heterogeneity may contribute to the different pathological outcomes of inflammation in the upper and lower airways.[42] It seems likely that the Th2 milieu upregulates NO production in the lower airway but downregulates NO production in the paranasal sinuses.

In summary, our results provide important detail that informs our understanding of the pathogenesis of CRS, raising the possibility that nasal NO levels may be useful as a marker of CRS severity. Specifically, we speculate that low nasal NO levels in patients with CRS might contribute to its pathogenesis, especially in the development of ECRS. In addition, we showed that nasal NO levels were significantly decreased in patients with CRS, especially in ECRS, and that this was not simply because of an obstructed middle nasal meatus or reduced nasal NO production through t-PA downregulation. Of interest, we also found a significant and negative correlation between the nasal NO levels and the JESREC score in patients with CRS (Fig. 6). In addition, the ROC curve analysis indicated that the optimal cut-off points of nasal NO levels are 77.3 ppb for discrimination of the all CRS patients and 53.0 ppb for discrimination of the ECRS patients, with sufficient sensitivity and specificity (Fig. 7). Therefore, we think that nasal NO measurement might be a simple, non-invasive, and useful clinical indicator of CRS recurrence and refractoriness. Finally, our results raise the exciting possibility that nasal NO induction may be developed as a novel therapeutic approach for CRS. Of course, these latter possibilities require further study.

## Acknowledgments

The authors would like to thank Hiroko Tsutiya (laboratory assistant, Division of Otorhinolaryngology Head and Neck Surgery, Department of Sensory and Locomotor Medicine, University of Fukui) for measurement of oral and nasal FeNO. The authors would like to thank Enago (www.enago.jp) for the English language review.

*Conflict of interest*

The authors have no conflict of interest to declare.

*Authors' contributions*

TT and SF designed and managed the research. KY, TT, MS,YI, and NN collected clinical data. KY and TT analyzed the data and wrote the manuscript. All authors revised the manuscript and approved the final version.

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