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ORIGINAL ARTICLE

Comparison of Nasal Nitric Oxide Levels between the Inferior Turbinate Surface and the Middle Meatus in Patients with Symptomatic Allergic Rhinitis

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ABSTRACT

Background: Because of the anatomical complexity and the high output of the human nose, it has been unclear whether nasal nitric oxide (NO) serves as a reliable marker of allergic rhinitis (AR). We examined whether nasal NO levels in the inferior turbinate (IT) surface and the middle meatus (MM) differ in symptomatic AR patients.

Methods: We measured fractional exhaled NO (FeNO) and nasal NO in normal subjects (n = 50) and AR patients with mild symptoms (n = 16) or moderate or severe symptoms (n = 27). Nasal NO measurements were obtained using an electrochemical analyzer connected to a catheter and an air-suction pump (flow rate 50 mL/sec).

Results: Compared to the normal subjects, the AR patients showed significantly higher nasal FeNO and nasal NO levels in the IT area. No significant difference in the MM area was observed among the three groups. The MM area showed higher NO levels than the IT area in all three groups. The ratio of nasal NO levels of the MM area to the IT area (MM/IT ratio) was significantly lower in the AR groups. The moderate/severe AR patients showed significantly higher nasal NO in the IT area (104.4 vs. 66.2 ppb) and lower MM/IT ratios than those in the mild AR patients. The analysis of nasal brushing cells revealed significantly higher eosinophil cationic protein and nitrotyrosine levels in the AR groups.

Conclusions: Nasal NO assessment in the IT area directly reflects persistent eosinophilic inflammation and may be a valid marker to estimate the severity of AR.

KEY WORDS

allergic rhinitis, exhaled nitric oxide, inferior turbinate, nasal nitric oxide, nitrotyrosine

ABBREVIATIONS

AR, allergic rhinitis; ECP, eosinophil cationic protein; FeNO, fractional exhaled nitric oxide; IT, inferior turbinate; MM, middle meatus; NO, nitric oxide; NT, nitrotyrosine; NOS, nitric oxide synthase; ppb, parts per billion.

INTRODUCTION

The standardization of measuring techniques by the American Thoracic Society/European Respiratory Society (ATS/ERS) has opened the way for the collection of comparable airway data on nitric oxide (NO) in normal subjects and those with disease states.[1,2] Allergic rhinitis (AR) has been considered to be asso-

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ciated with increased NO levels. However, it has not yet been determined whether nasal NO serves as a reliable index of disease severity, or to what extent NO concentrations inside the nose contribute to pathologies of AR.[3-5] Nasal NO is not routinely measured in daily clinical practice. One reason may be the heterogeneous results found in previous studies, mainly due to the complexity of the anatomical and physiological features of the human nose and the lack of consensus concerning the suitable measurement technique.[6-8]

In the present study, the fractional exhaled nitric oxide (FeNO) and nasal NO levels were examined in a population of normal subjects and AR patients. We used a newly designed method to measure nasal NO locally based on the anatomical features of the nasal cavity. For this purpose, the NO analyzer was connected to a suction catheter and a fixed-quantity suction pump in an out-patient clinic setting. The handheld device with an electrochemical sensor for nasal NO measurement has been shown to be reliable and simple to use at a lower cost.[9,10]

Here we examined whether local gradients of NO concentration in different areas inside the nasal cavity differ among normal subjects and AR patients classified by subjective symptom severity. We also obtained nasal brushing cells from the surface of the inferior turbinate mucosa and analyzed the concentrations of the extracted inflammatory mediators related to eosinophil activation and NO metabolism. We note that the nasal NO assessment described here is noninvasive, quickly performed, and may directly reflect the degree of allergic inflammatory conditions adjacent to the surface mucosa of the inferior turbinate.

METHODS

SUBJECTS

Ninety-three subjects were included in this cross-sectional, between-group and method comparison study. The subjects were 43 patients with perennial AR without bronchial asthma (28 males and 15 females; mean age 27.7 years) and 50 normal volunteers without nasal symptoms (30 males and 20 females; mean age 32.1 years). The AR patients were recruited on an outpatient setting and subdivided into two groups based on disease severity: the group of 16 patients with mild symptoms (mild group) and the group of 27 patients with moderate or severe symptoms (moderate/severe group).

The diagnosis of AR was based on clinical history, the presence of subjective nasal symptoms together with positive nasal eosinophil tests, and positive skin reactions or serum allergen-specific IgE antibody measurements against house dust mites. Nasal endoscopy was performed for all subjects before measuring nasal NO in order to assess the degree of nasal septum deviation and patency of middle meatus, and to exclude the presence of nasal polyposis. We excluded current-smokers and patients who had been treated with any allergen-specific immunotherapy. The patients did not receive any anti-allergic medication in the 30 days before the study. The patients' subjective symptoms were recorded at the time of the NO measurement. They include the average number of paroxysmal sneezing, episodes of nose blowing, and the degree of nasal blockage. The severity of the disease was then determined as mild, moderate or severe based on the classification proposed by the Japanese guideline for allergic rhinitis.[11]

The study protocol was approved by the Institutional Review Board at the Hiroshima University School of Medicine (Project approval #181-1). The purpose of the research and experimental protocols was explained to all participants, and written informed consent was obtained prior to the study.

NITRIC OXIDE MEASUREMENTS

The subjects' NO levels were measured using a handheld electrochemical analyzer (NObreath®, Bedfont Scientific, Rochester, UK) according to the ATS/ERS guidelines.[1] For the oral FeNO measurements, the subjects exhaled at a flow rate of 50 mL/s through a mouthpiece assisted by visual cues. For the nasal FeNO measurements, the subjects were instructed to exhale transnasally with their mouth closed into a nose adaptor at the same flow rate, as described.[12] The nasal FeNO measurements were carried out for the right and left nasal cavities separately, with the other nostril closing in turn. We also measured nasal NO in all of the subjects by directly aspirating air from the nasal cavity. For this purpose, the NO analyzer was connected to a suction catheter via a sterile syringe filter and a portable air-suction pump (MP-Σ300N, Sibata Science, Saitama, Japan), which could be set at constant flow levels (Fig. 1). The aspiration flow rate was fixed at a rate of 50 mL/sec, and the tip of the catheter was placed inside the nasal cavity under direct vision during the sampling period. Two different target areas were set based on the anatomical features of the nasal cavity, i.e., near the anterior surface of the inferior turbinate (IT area) and the front of the middle meatus (MM area). The subjects were advised to breathe through the mouth with their soft palate elevated to cease the choanal airflow. Nasal NO levels were measured separately for the left and right side, leaving the other nostril open, in alignment with ATS recommendations.[1] The measurements were performed in the same clinic under constant environmental conditions. The measurement was repeated three times and the mean value was used for analysis.

NASAL CELL SAMPLING

Nasal brushing cell specimens were obtained for an enzyme-linked immunosorbent assay (ELISA) from 27 of the 50 normal subjects and 31 of the 43 AR pa-

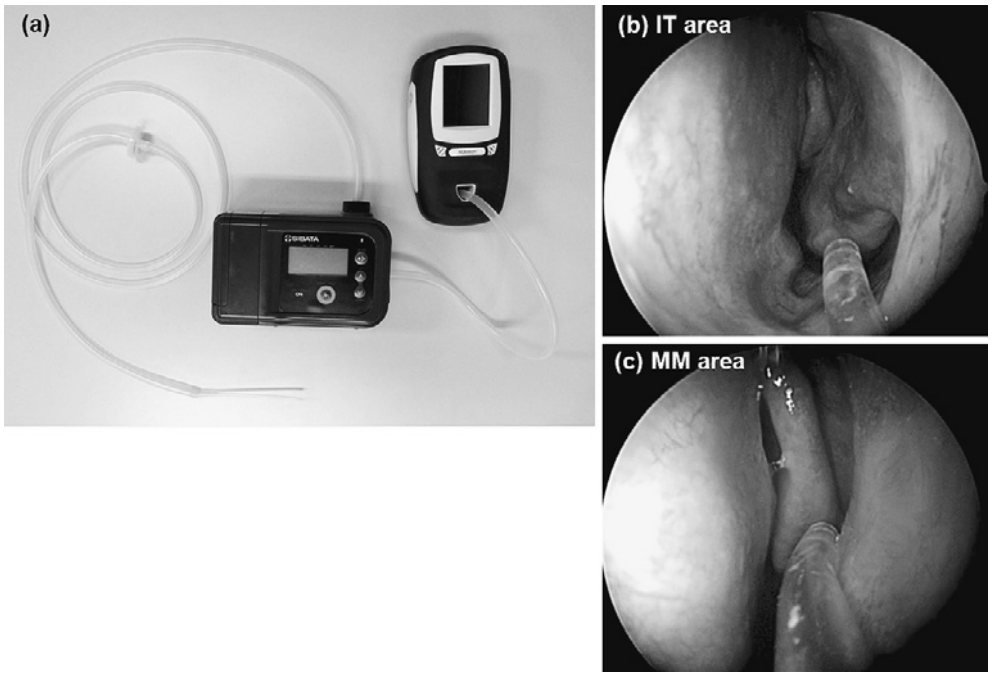

Fig. 1 (**a**) Configuration of the nose catheter, silicon tubes, and the air-suction pump used for the direct nasal NO measurement. The tip of the catheter was placed (**b**) in the anterior surface of the inferior turbinate (IT) or (**c**) in front of the middle meatus (MM) during the aspiration period.

tients (10 in the mild group and 21 in the moderate/severe group) who agreed to participate. There was no artificial bias for patient selection in cell sampling. A topical decongestant was applied to the nose just after the completion of the series of NO measurements, and secretions were carefully removed. Cells were then obtained by scraping of the medial wall of the IT under direct vision using a Cytobrush® (Medscand Medical, Malmo, Sweden). The cells were placed immediately in a 2-mL volume of chilled phosphate buffered saline (PBS) and then stored at -80℃ until further use. Protein extraction from the cell suspension was performed by a tissue homogenizer using bead-beating technology (Precellys® 24, Bertin Technologies, Montigny-le-Bretonneux, France) in 2-mL tubes with 1.4-mm prefilled glass beads (6000 rpm, two cycles for 20 s each).

The concentrations of eosinophil cationic protein (ECP) and nitrotyrosine (NT) from the supernatant were measured quantitatively by the ELISA method. Commercially distributed kits for ECP (ECP ELISA, Aviscera Bioscience, Santa Clara, CA, USA) and for NT (OxiSelect™ Nitrotyrosine ELISA Kit, Cell Biolabs, San Diego, CA, USA) were used according to the instructions supplied by the manufacturer.

DATA ANALYSIS

Group data are expressed as means ± standard deviations (SD). For multiple comparisons, a screening of data for differences was first carried out using the Kruskal-Wallis test. If the analysis gave a significant result, a further comparison was done by the Mann-Whitney U-test for the between-group analysis. The comparison of paired nasal NO levels between different areas was assessed with the Wilcoxon rank sum test. Spearman rank correlation was used in evaluating correlations. *P*-values < 0.05 were considered significant.

RESULTS

DIRECT MEASUREMENT OF NASAL NO LEVELS

The demographics and clinical background of the study population are summarized in Table 1. No significant difference between the normal and AR groups was found in the baseline data of gender or age distribution. The distribution of the oral and nasal FeNO values in each group is shown in Figure 2. Compared to the normal subjects, the patients in both AR groups showed significantly higher levels of nasal FeNO. There was no significant difference in nasal FeNO levels between the mild and the moderate/severe AR groups. The AR patients tended to show higher levels of oral FeNO, but the difference was not significant. The direct measurement of nasal NO levels from the IT area and the MM area inside the nasal cavity was successfully achieved using the current setting for all of the subjects. We had difficulty measuring the nasal NO levels in the MM area of the unilateral side in four normal subjects and three AR patients because of severe nasal septum deviation.

Table 1 Demographics and clinical background of the study population

	Normal volunteers	AR patients	
		Mild group	Moderate/severe group
number (male/female)	50 (30/20)	16 (11/5)	27 (17/10)
age	32.1 (12.5)	26.1 (5.8)	28.7 (8.4)
subjective nasal symptom score			
sneezing	-	0.31 (0.47)	1.48 (1.08)
nose blowing	-	0.81 (0.4)	2.26 (1.09)
nasal blockage	-	0.87 (0.34)	2.44 (1.05)
total score	-	2 (0.63)	6.19 (2.76)

Data are shown as mean with standard deviations in parenthesis.

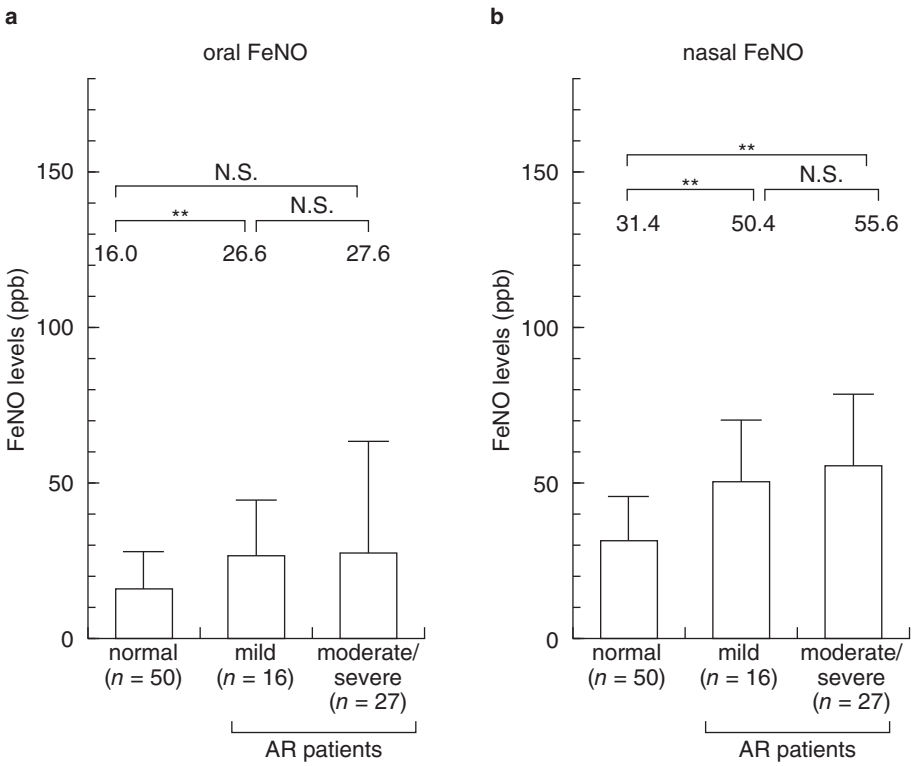

Fig. 2 (**a**) Oral and (**b**) nasal FeNO levels in the normal subjects (n = 50) and AR patients in the mild group (n = 16) and in the moderate/severe group (n = 27). The average of right and left nasal cavities was used for the nasal FeNO level of each individual. Error bars = mean values and SD. *$p < 0.05$; **$p < 0.01$; N.S., no significance; FeNO, fractional exhaled nitric oxide.

Therefore, 96 sides of the nasal cavity in the normal group and 83 sides in the AR group were processed for analysis. None of the subjects reported adverse effects after the procedure.

As shown in Figure 3, the nasal NO levels in the IT area in both groups of AR patients were significantly higher than those of the normal subjects. The AR patients in the moderate/severe group also showed significantly higher nasal NO in the IT area compared to the mild AR patients (104.4 ppb vs. 66.2 ppb). However, there was no significant difference in nasal NO levels in the MM area among the three groups. Consequently, the ratio of nasal NO levels of the MM area to the IT area (MM/IT ratio) was significantly lower in the two AR groups than in the normal group (Fig. 3c), with the moderate/severe AR group showing significantly lower MM/IT ratios compared to the mild AR group. When the same nasal cavity was compared for each subject, the MM area showed higher NO levels than the IT area, and the differences were

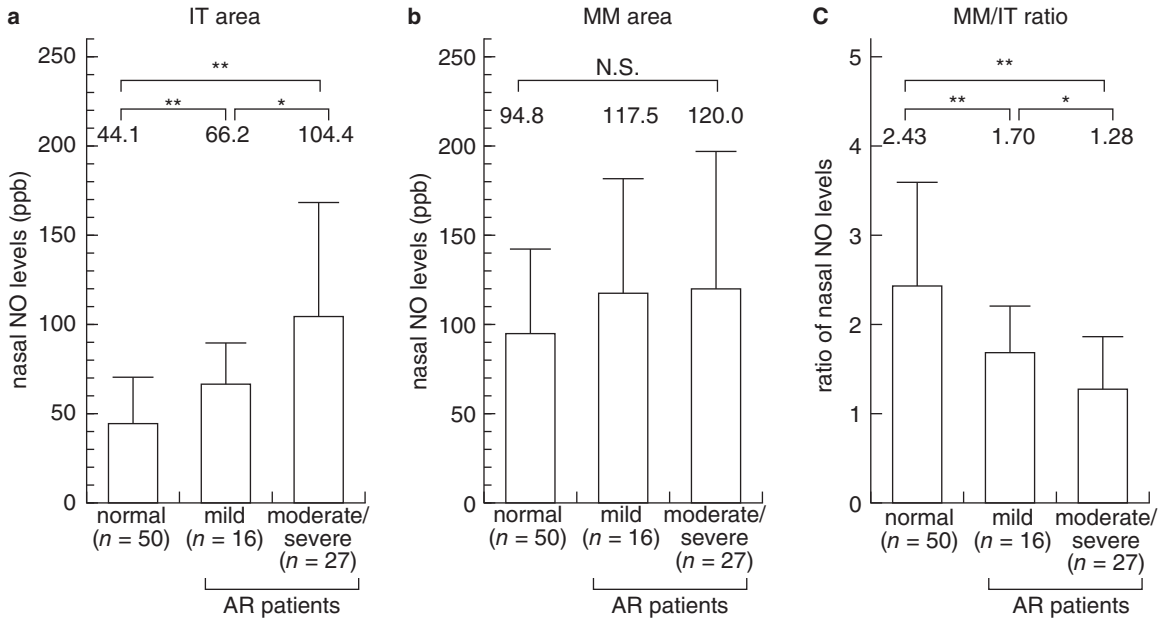

Fig. 3 Nasal NO levels (**a**) in the IT area and (**b**) in the MM area in the normal subjects and AR patients in the mild and moderate/severe groups. (**c**) The nasal NO ratio of the MM area to the IT area (MM/IT ratio) for each group. Measurement of the nasal NO levels and the calculation of the MM/IT ratio were carried out separately for the left and right sides of the nose, and the average of the two cavities was used for each individual. Error bars = mean values and SD. *$p < 0.05$; **$p < 0.01$; N.S., no significance.

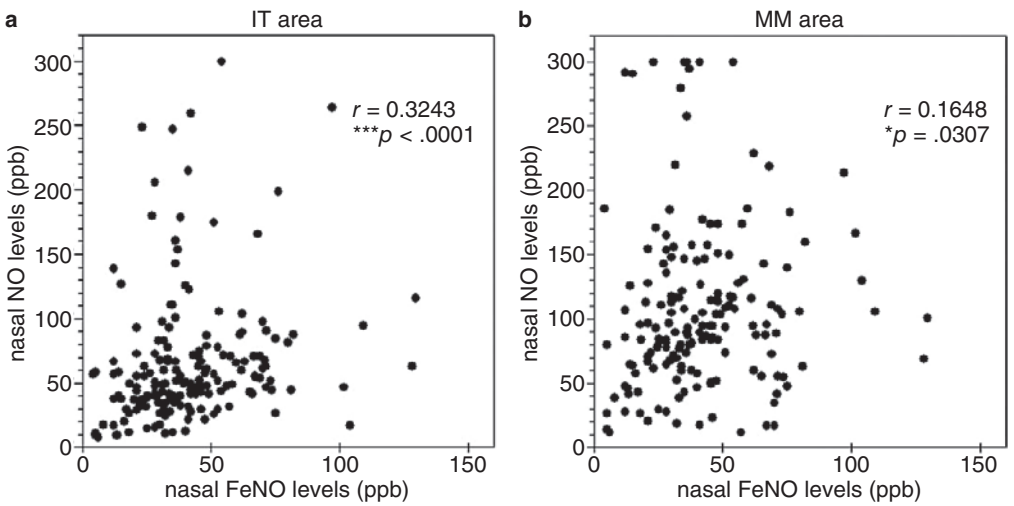

Fig. 4 Correlation between nasal FeNO levels and nasal NO levels (**a**) in the IT area and (**b**) in the MM area for each nasal cavity in all subjects ($n = 179$).

significant in all three groups. The mean differences in the nasal NO levels in the IT area between the right and left cavities were 16.1 ppb (SD 21.1) in the normal group, 18.9 ppb (SD 10.8) in the mild AR group, and 39.7 ppb (SD 35.7) in the moderate/severe AR group. The mean differences in the nasal NO levels between the cavities were more pronounced in the MM area: 30.5 ppb (SD 33.8) in the normal group, 38.5 ppb (SD 42.5) in the mild AR group, and 35.4 ppb (SD 35.1) in the moderate/severe AR group. The correlations between nasal FeNO levels and nasal NO levels in the IT and MM areas for each nasal cavity are shown in Figure 4. We found positive correlations between the paired two parameters, but the coefficient was markedly higher for nasal NO in the IT area than that in the MM area ($r = 0.3243$ vs. $r = 0.1648$).

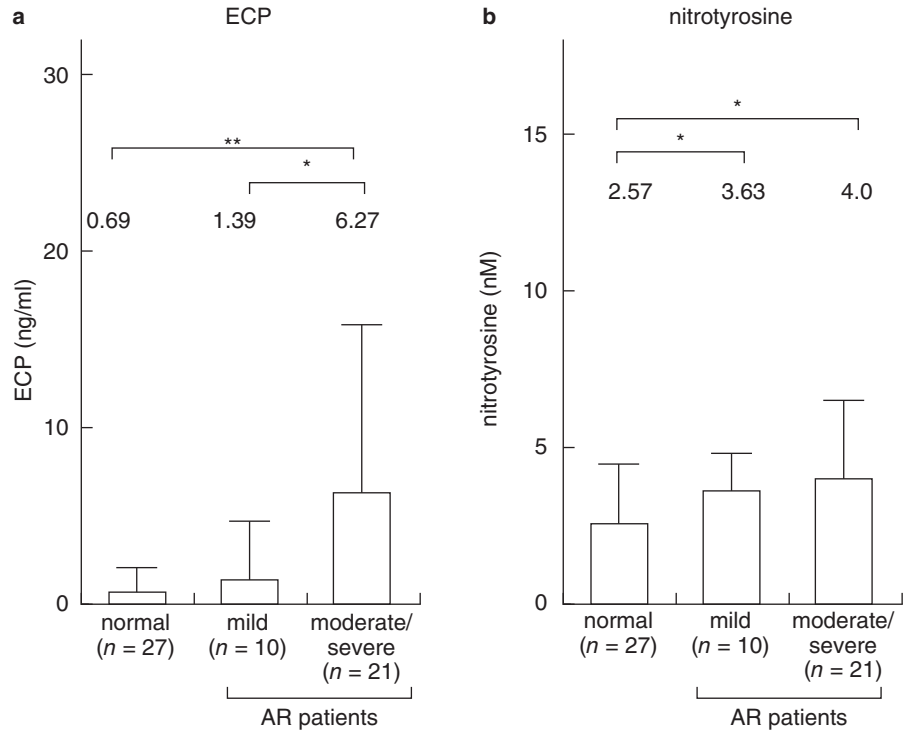

Fig. 5 Comparison of the levels of (**a**) ECP and (**b**) nitrotyrosine in the nasal brushing cells. Error bars = mean values and SD. *$p < 0.05$; **$p < 0.01$.

ELISA OF NASAL BRUSHING CELLS

The ELISA of nasal brushing cells revealed elevated concentrations of ECP derived from activated eosinophils and nitrotyrosine from oxidized NO metabolites in the allergic patients (Fig. 5). The mean ECP level in the moderate/severe group was significantly higher than that in the normal subjects. The mean NT levels in the two AR groups were both significantly higher than that in the normal group.

DISCUSSION

Allergic rhinitis has been thought to be associated with increased NO levels, mainly by the increased inducible nitric oxide synthase (iNOS) expression in the inferior turbinate mucosa.[5,13-15] Several authors have reported increased nasal NO levels in symptomatic AR patients.[12,16-20] Conversely, some studies detected no significant differences between AR patients and control subjects.[21-23] These seemingly conflicting results regarding NO levels may reflect the functional complexity of NO in the human nose. There is a great difference in background NO output between the upper and the lower airways. In the upper airways, there is a high background output, and thus an increase (e.g., allergic rhinitis) tends to be obscured, whereas a decrease (e.g., primary ciliary dyskinesia or chronic rhinosinusitis with nasal polyps) is usually easier to reveal.[8,24] Although NO production in the nasal mucosa of AR patients may be up-regulated, this increase could be counteracted by swelling of the mucosa and secretions that lead to impaired NO diffusion. In addition, the high background levels of NO from constitutive sources may blunt smaller increases in mucosal NO output. We hypothesize that nasal NO is a valid marker for allergic inflammation and that the measurement of local NO levels based on the anatomical features of the human nasal cavity might be useful in distinguishing AR patients from normal subjects. High nasal NO levels detected in the IT area in symptomatic AR patients can directly reflect the persistence of mucosal eosinophilic inflammation in the inferior turbinate.

In this study, we validated two different methods as recommended by the ATS to assess the NO level in the nasal cavity, i.e., nasal FeNO and nasal NO.[1] Nasal FeNO is considered to represent a fraction of endogenous NO with contaminated air passing through the nose at a relatively high flow rate.[7] The exhalation process may be influenced by changes in the airflow physics caused by inter-individual variations in the anatomical structure of the nasal cavity. As for nasal NO measurements, most of the previous reports concluded that nasal NO can be measured with fair reproducibility based on several different approaches proposed thus far.[17,25,26] Qian *et al*. found no significant difference in nasal NO with aspiration flows ranging from 2.2 to 6.2 L/min, which is in agreement with the recommended aspiration by the ATS.[27] In

the present study, the nasal NO measurement was conducted by direct sampling from one side of the nose with an airstream of a constant rate of 3 L/min. The subjects were instructed to breathe orally with their soft palates elevated and to block the communication to the nasal cavity. The method enables us to avoid possible contamination of exhaled NO derived from the lower respiratory tract, and the results obtained are immediately available for clinical assessment.

The results of the present study clearly indicate that increased NO levels near the surface of the inferior turbinate can be a simple and sensitive marker for the diagnosis of AR. In order to avoid an impact on the nasal NO results due to a sizable contribution from the paranasal sinuses, we found it advantageous to perform the monitoring in this designated area. The nasal FeNO levels in this study were significantly different between the AR patients and normal controls. This finding is compatible with our previous study, although the distribution of nasal FeNO levels in the present population was shifted slightly lower.[12] One possible explanation for this change is that the nasal FeNO measurements were carried out for the right and left nasal cavities separately in this study, whereas transnasal exhalation procedure was done bilaterally in our previous study. These results seem to be related to an intimate association between nasal NO in the IT area and nasal FeNO irrespective of allergic diathesis, suggesting that the aerodynamic distribution of NO levels in the human nose is a continuous trait.[28] However, the discriminative power of nasal NO in the IT area in the present study was higher than that obtained with conventional FeNO measurement techniques. We found that the severity of daily nasal symptoms in the AR patients was reflected as an increase in nasal NO levels in the IT area. Although our statistical analysis indicated close correlations between nasal FeNO levels and nasal NO levels in the IT area, some of the subjects had shown relatively higher nasal NO values in the IT area. The tendency was more pronounced in the moderate/severe AR patients. The reasons for this phenomenon are unclear, but several previous studies indicate the same tendency.[4,6] The exhalation process in the moderate/severe AR patients with hypertrophic inferior turbinates may be influenced by modification of the nasal airflow in narrowing pathways.

As for the difference in nasal NO between the right and left nasal cavities, Alexanderson *et al*. reported that in 331 normal and symptomatic subjects, the mean difference in nasal NO between the nostrils was 14 ppb and nearly 95% of the subjects had a difference of <45 ppb.[26] They also found that atopy was significantly associated with a high difference of nasal NO levels between the nostrils. These results are consistent with those of the present study. We also found that 95.6% (44/46) of the normal subjects, 93.3% (14/15) of the mild AR patients, and 68% (17/25) of the moderate/severe AR patients had a difference of <45 ppb in the IT area. These findings indicate that large differences in nasal NO between the cavities may predict the presence of not only atopic diathesis but also an ongoing inflammatory allergic reaction in the inferior turbinate. Another supplementary finding of this study is that the difference in nasal NO levels between the IT area and the MM area can also be used as a marker for AR diagnosis. The MM/IT ratio was significantly lower in the AR group, with the differences being more significant than the FeNO measurement. However, limited data are available for the interpretation of the MM/IT ratio in the present study, because we performed the nasal endoscopy only to evaluate the patency of the middle meatus. Further studies are necessary for the use of this parameter to evaluate various nasal diseases (including AR) in relation to inter-individual differences in anatomical characteristics of the nose.

Nasal NO levels in the MM area have been postulated to depend mainly on the amount of NO produced by the ciliated epithelium of the paranasal sinuses and the size of the paranasal sinus ostia.[29] In the present study, we found that most participants showed higher NO levels in the MM area than in the IT area (94.8% of the normal subjects and 74.7% of the AR patients). By emphasizing the MM area in this study, we assumed that the maxillary and anterior ethmoid sinuses are the dominant source of nasal NO detected in this area. Arnal *et al*. also found that symptomatic AR patients exhibited an acute increase in nasal NO after the administration of a topical vasoconstrictor, suggesting that acute changes in the widening of the ostio-meatal complex may affect the physiological mixing of paranasal sinus NO with that of the nasal cavity.[18] However, since standardized measurements of sinus NO have not yet been established, further studies are required to assess the relative importance of the volume-surface area of the individual sinus in relation to the NO transport to the nasal cavity, where it is commonly measured.

The increased NO in allergic rhinitis has several pathophysiologic consequences, including vasodilation, modification of sensory nerve endings, and accumulation of activated eosinophils. Nitrotyrosine is a stable downstream product of multiple pathways formed in the presence of excess NO production and oxidative stress by the modification of tyrosine residues.[30,31] In patients with severe asthma, the degree of airway eosinophilia can be predicted by a combination of FeNO levels, iNOS expression, and NT production.[32,33] The intranasal administration of eotaxin to AR patients also induced a significant increase in nasal NO with accompanying local eosinophil recruitment.[34] In the present study, the production of both ECP and NT was significantly up-regulated in the AR patients, associated with elevated nasal NO in the IT

area. These results are in accord with those of these previous studies, where the NO concentration was thought to reflect eosinophilic inflammation and autotoxic NO metabolisms.

The limitations of this study include its cross-sectional study design, without ability to see the effects of therapeutic modalities for each patient. Further studies are required to reinforce the usefulness of nasal NO measurement as an objective method for assessing the outcome of various anti-allergic therapies.

The on-line method for nasal FeNO and nasal NO measurements is highly reproducible and easy to perform for both the subject and the clinician. Increased NO levels near the IT surface can be sensitive markers for the diagnosis of allergic rhinitis, with the significance being more prominent than nasal FeNO. Relatively high nasal NO levels in the MM area also indicate the role of paranasal sinuses acting as a physiological NO reservoir in humans. We suggest that such advances in the standardized measurement techniques and established guidelines for standard values will exploit nasal NO for the diagnosis, treatment, and monitoring of relevant upper airway disorders.

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