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ORIGINAL ARTICLE

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The Relationship between Exhaled Nitric Oxide Measured with an Off-line Method and Airway Reversible Obstruction in Japanese Adults with Asthma

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ABSTRACT

Background: Exhaled nitric oxide (eNO) is a useful marker of eosinophilic airway inflammation in asthma patients. There is no study to show the relationship between the eNO measured by using an off-line method and the degree of reversibility of airflow limitation in Japanese asthma patients. We sought to investigate the relationship between the eNO level measured by using an off-line method and the degree of reversibility of bronchial constriction in Japanese asthma patients.

Methods: The study population comprised 97 asthma patients in our outpatient clinic with some patients in both groups who received inhaled corticosteroid treatment. We measured eNO levels, forced expiratory volume in one second (FEV_1) before and after treatment, reversible airway obstruction (ΔFEV_1) after inhalation of bronchodilator, and other parameters.

Results: eNO was significantly correlated with peripheral blood eosinophil counts in asthma patients (in steroid-naïve asthma patients, $r = 0.544$, $p < 0.0001$; in asthma patients treated with inhaled corticosteroid, $r = 0.463$, $p = 0.026$), and subjects with severe eosinophilia in sputum showed high levels of eNO (mild eosinophilia *versus* severe, $p = 0.0152$). Among patients with obstructive impairment, eNO levels were correlated with ΔFEV_1 regardless of whether patients received ($r = 0.527$, $p = 0.0435$) or did not receive ($r = 0.64$, $p = 0.0056$) inhaled corticosteroid. In subjects with normal pulmonary function, there was no significant relationship between eNO and ΔFEV_1 with or without inhaled corticosteroid.

Conclusions: In patients with obstructive impairment, eNO reflects the degree of reversible airflow limitation. In subjects with normal pulmonary function, eNO may facilitate the diagnosis and management of asthma, rather than indicate reversible bronchial obstruction. eNO measurement by off-line methods is applicable as a potential tool for the diagnosis of asthma and management of asthma patients.

KEY WORDS

airflow reversible obstruction, asthma, exhaled nitric oxide, off-line method

INTRODUCTION

Bronchial asthma is caused by eosinophilic bronchial inflammation. Inhaled corticosteroid (ICS), the mainstay of asthma treatment, is effective because it prevents this inflammatory process. Therefore, quantifi-

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cation of airway inflammation may provide additional information for both diagnosis and management of bronchial asthma. However the current asthma management guideline (GINA: Global Initiative for Asthma) relies on monitoring respiratory function and symptoms.[1] Three studies have demonstrated that adding alternative monitoring markers such as airway hyperresponsiveness, eosinophilia in induced sputum, and exhaled nitric oxide (eNO) to current guidelines on dose adjustment of ICS leads to improved outcomes.[2-4]

According to recent studies, eNO is a useful marker of airway eosinophilic inflammation in asthma.[5,6] The increased levels of eNO are due to activation of NO synthase in airway epithelial and inflammatory cells.[7-9] Measurement of eNO is simple, noninvasive, and repeatable. eNO levels are higher in asthma patients than in healthy subjects,[10] and eNO levels fall after treatment with corticosterids.[11] In steroid-naïve asthma patients, eNO levels correlate with peripheral eosinophils in blood, eosinophils in induced sputum, airway reversible limitation, or bronchial hyperresponsiveness,[12-16] so that eNO is useful in the diagnosis of asthma patients.[17,18] In asthma patients treated with ICS, eNO levels correlate with peripheral eosinophils in the blood, but are not correlated with eosinophils in induced sputum or bronchial hyperresponsiveness.[19] However, eNO levels correlate with the following markers of disease control: asthma symptoms within the prior 2 weeks, disease score, and reversibility of airflow obstruction of bronchial asthma.[20] Furthermore, eNO is useful in the clinical management[4] of asthma patients treated with ICS. These findings suggest that eNO is not only useful in steroid-naïve asthma patients, but also useful as a marker of disease control in asthma patients treated with ICS.

Despite the utility of the eNO measurement, NO analyzers are too expensive for widespread use by general practitioners. In comparison, the off-line (bag collection) method of eNO measurement may be more useful.[5,6,21] In contrast to on-line techniques, off-line collection methods offer the following benefits: (1) the potential for expirate collection at sites remote from the analyzer; (2) more efficient use of the analyzer because exhaled gas can be collected and stored for a while from several patients or several places and analyzed in a batch, possibly with notable cost savings. Recent studies have demonstrated that eNO measurement in the off-line method has sensitivity and specificity in which is comparable to the on-line method,[22-25] especially in asthmatic children. However in Japan, there have been few studies on the use of eNO in asthma patients using the off-line method. Therefore, we sought to investigate the relationship between eNO levels measured by using an off-line method and the level of bronchial reversibility in Japanese asthma patients.

METHODS

SUBJECTS

The study population was recruited from adult outpatients with bronchial asthma (n = 97) at the Clinic of Allergy and Respiratory Medicine in the Sagamihara National Hospital from June 2003 until February 2005. All patients gave full informed consent to participate in the study. Each subject underwent a standard clinical assessment, which included a history and physical examination, laboratory tests (including eosinophil counts in peripheral blood), and chest radiograph. The diagnosis of asthma was based on application of the GINA guidelines by an experienced respiratory physician blinded to the result of eNO measurement.[1] The clinical severity of asthma (Step) was classified according to these guidelines.[1] Atopy was indicated by a positive skin test to mites or housedust, or serum IgE >250 IU/ml. Exclusion criteria included current smokers or ex-smokers of greater than 20 packs per year, rescue use of oral corticosteroids within the preceding four weeks, pregnancy, and any other respiratory disease. The study protocol (no. 14, 2003) was approved by the ethical committee at our hospital .

eNO MEASUREMENT

Exhaled air was collected according to the recommendation of ATS/ERS by using bag collection kits (Sievers Instrument, Boulder, Colo).[5,6] Briefly, subjects inhaled deep breaths of room air through the NO scavenging filter and exhaled through a mouthpiece with a flow rate of 70 ml per second against an expiratory resistance of 10 cm H_2O and, 5 seconds later, exhaled air was collected into the 1.5 liter Mylar bag provided in the kit. The NO concentration in the collected exhaled air was measured within 12 hours. The air was drawn out of the balloons at 200 ml per minute into an NO chemiluminescence analyzer (NOA model 280A, Sievers Instrument) with a response time of 200 milliseconds. The normal range of eNO was determined in 14 healthy non-atopic, nonsmoking volunteers. Their mean value was 21.1 ppb; the 95%CI was 15.0–27.1 ppb. Regarding this range, we considered basal concentrations≧27.2 ppb as the cutoff values.

SPIROMETRY

After the eNO samples were collected, all subjects underwent spirometry with an electric spirometer (Minato Autospiro AS-302, Japan). The forced expiratory volume in 1 second (FEV_1) was expressed as percentage of forced vital capacity (FVC). The reversible change of FEV_1 was expressed before and 30 minutes after inhaling 200 μg salbutamol. The degree of reversible airflow limitation was defined as ΔFEV_1, which is equal to $(FEV_{1post\text{-}bronchodilator} - FEV_{1baseline}) \times 100/FEV_{1baseline}$ (%).

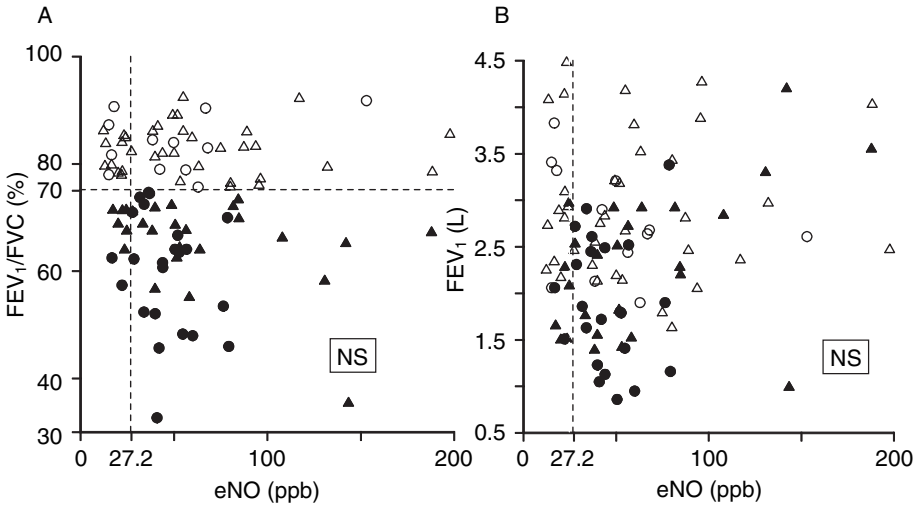

Fig. 1 Correlation between eNO and FEV_1/FVC (%) (**A**) or FEV_1 (L) (**B**). Triangles, asthma patients treated without inhaled corticosteroid (ICS); Circles, asthma patients treated with ICS.; Open symbols, $FEV_1/FVC \geqq 70\%$; Solid symbols, $FEV_1/FVC < 70\%$; NS, not statistically significant. The vertical line represents $FEV_1/FVC = 70\%$, and the horizontal line represents eNO = 27.2 ppb.

Table 1 Patient demographics. Data are presented as mean ± standard error of the mean.

	Bronchial asthma	
	With ICS	Without ICS
Sex (Men/Women)	11/24	23/39
Age (year)	48.6 ± 2.68	40.9 ± 1.93
Atopy/non-atopy	26/9	42/19
Duration of disease (year)	12.5 ± 2.41	5.68 ± 1.07
Step 1	6	23
2	9	13
3	14	19
4	6	6
FEV_1/FVC (%)	68.1 ± 2.51	75.5 ± 1.33
$\%FEV_1$ (%predict)	81.6 ± 3.87	93.0 ± 2.18
Eosinophil in blood (%)	7.02 ± 1.00	6.46 ± 0.63
Eosinophil in sputum (mild/moderate/severe)	(1/0/6)	(10/8/21)
eNO (parts per billion; ppb)	46.9 ± 4.37	61.7 ± 5.70
$DFEV_1$ (%)	13.5 ± 0.13	7.98 ± 0.12
median	9.8%	6.5%

EOSINOPHILIA IN SPUTUM

Sputum was collected and analyzed using the Hansel's modified method (Eosinostein©, Torii Pharmaceutical Co., Tokyo, Japan).[26] Briefly, each sputum sample was smeared on a glass slide and stained for 45 seconds. All samples were analyzed by an experienced investigator (A.S.) who was blinded to the clinical data of the patients. The level of eosinophilia was scored as: mild, no or few eosinophils throughout the entire smear; moderate, several eosinophils in each high-power (×400) microscopic field; severe, numerous eosinophils in each high-power (×400) microscopic field.

STATISTICAL ANALYSIS

Correlations were determined using the Spearman rank correlation. A paired-sample *t* test was used to analyze eNO measurements and FEV_1, ΔFEV_1 or peripheral eosinophil percentage in blood; $p < 0.05$ was considered significant. Analysis of variance was used to compare eNO measures between groups according to the level of eosinophilia in the sputum.

RESULTS

We classified the 97 asthma patients into two groups in regard to their therapeutic backgrounds: (A) 35 asthma patients treated with ICS (ICS(+)) and (B) 62 stable asthma patients treated without ICS (ICS(–)) (Table 1).

There was no relationship between eNO levels and either FEV_1 (L) or FEV_1/FVC (%) among the patients in either groups (Fig. 1A, B). The peripheral eosinophil percentage in blood was correlated with the eNO levels in each group (steroid-naïve asthma patients, $r = 0.544$, $p < 0.0001$, Fig. 2A; asthma patients with ICS, $r = 0.463$, $p = 0.026$, Fig. 2B).

Sputum was collected from 7 of the 35 subjects

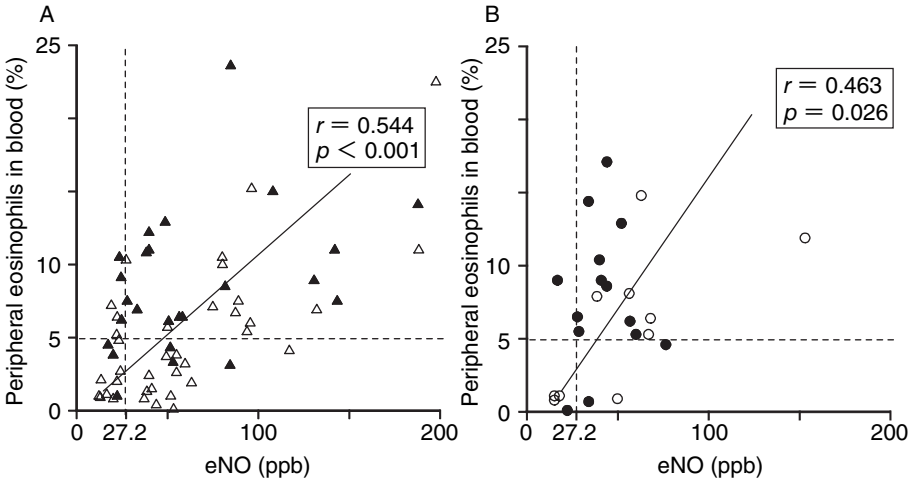

Fig. 2 Correlation between eNO and peripheral eosinophil percentage in blood. (**A**) asthma patients treated without ICS (triangles). (**B**) asthma patients treated with ICS (circles). Open, $FEV_1/FVC \geq 70\%$; Solid, $FEV_1/FVC < 70\%$. The vertical line represents peripheral eosinophilia in blood = 5%, and the horizontal line represents eNO = 27.2 ppb.

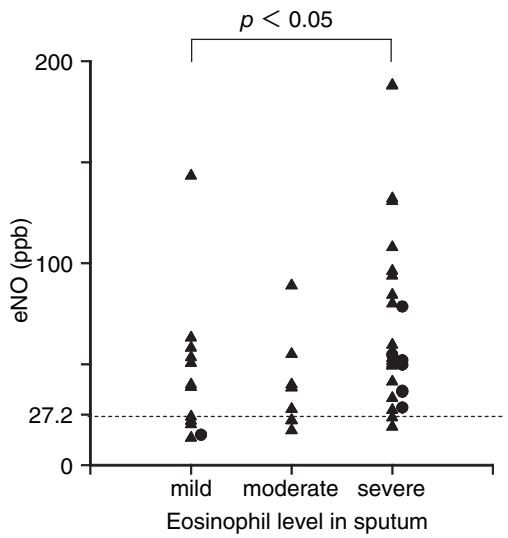

Fig. 3 The amounts of eNO at each level of eosinophilia in the sputum. Triangles, asthma patients treated without ICS; Circles, asthma patients treated with ICS. The horizontal line represents eNO = 27.2 ppb.

(20%) in the ICS (+) group and from 39 of the 62 subjects (62.9%) in the ICS (–) group. The eNO levels of the subjects with severe eosinophilia in the sputum were significantly (p = 0.0152) higher than those of patients with no or mild eosinophilia in the sputum (Fig. 3).

To investigate whether the level of airway eosinophilic inflammation influences the degree of obstructive impairment, we classified each of the ICS (+) and ICS (–) groups into two subgroups according to the results of the respiratory function tests: (1) asthma patients with airflow limitation in the respiratory function test ($FEV_1/FVC < 70\%$) and (2) asthma patients with normal function ($FEV_1/FVC \geq 70\%$).

Among the patients with obstructive impairment, eNO levels were correlated with ΔFEV_1 in the ICS (–) subjects (group A, r = 0.64, p = 0.0056, Fig. 4A). In addition, eNO levels were also correlated with ΔFEV_1 in the ICS (+) subjects (group B, r = 0.527, p = 0.0435, Fig. 4B). For subjects with normal pulmonary function, there was no significant relationship between eNO levels and ΔFEV_1 in either the ICS (–) (Fig. 5A) or the ICS (+) (Fig. 5B) subjects. The eNO levels of 24 out of 36 ICS (–) subjects or the eNO levels of 10 out of 17 ICS (+) subjects were higher than 27.2 ppb despite the low levels of ΔFEV_1.

DISCUSSION

In asthma patients with airflow limitation who were treated either with or without ICS, eNO levels were significantly associated with ΔFEV_1. However, in asthma patients with normal respiratory function who were treated either with or without ICS, there was no significant relationship between eNO and ΔFEV_1. In mild asthma conditions, there is eosinophilic inflammation of bronchial mucosa, even if the patient's respiratory function is normal. This might explain why there was no significant relationship between eNO and ΔFEV_1 in asthma patients with currently normal respiratory function. Furthermore this suggests that the biomarker reflecting airway inflammation can be useful for diagnosis and disease control in asthma patients.

eNO levels and the percentage of eosinophils in the peripheral blood are significantly correlated, and

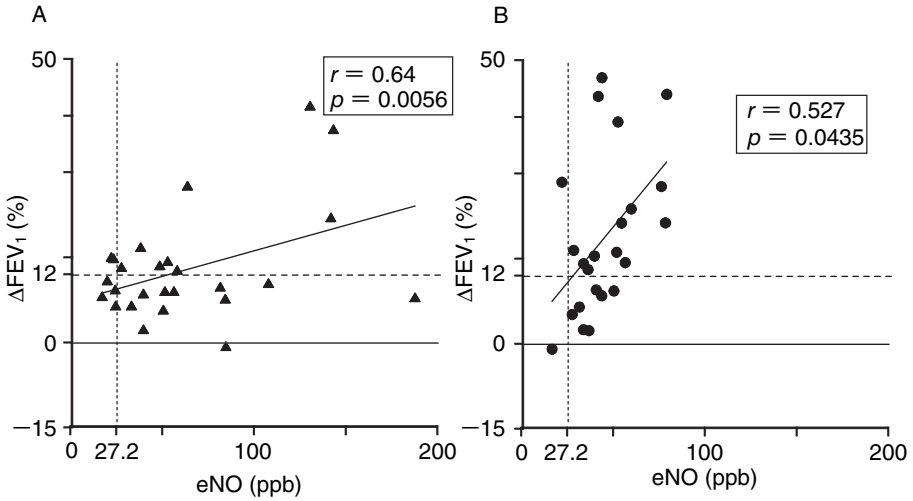

Fig. 4 Correlation between eNO and ΔFEV_1 (%) in asthma patients with obstructive impairment. (**A**) asthma patients treated without ICS (solid triangles). (**B**) asthma patients treated with ICS (solid circles). The vertical line represents $\Delta FEV_1 = 12\%$, and the horizontal line represents eNO = 27.2 ppb.

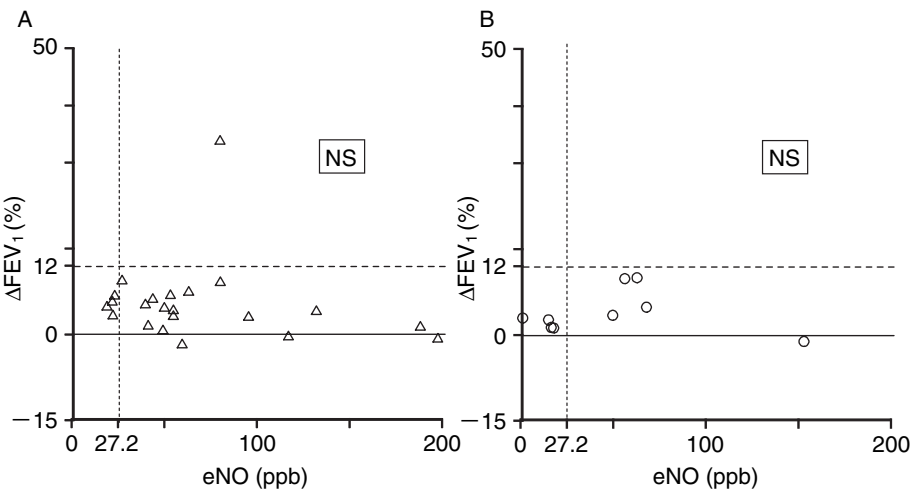

Fig. 5 Correlation between eNO and ΔFEV_1 (%) in asthma patients with normal respiratory function. (**A**) asthma patients treated without ICS (open triangles). (**B**) asthma patients treated with ICS (open circles). The vertical line represents $\Delta FEV_1 = 12\%$, and the horizontal line represents eNO = 27.2 ppb.

subjects with severe eosinophilia in sputum show high levels of eNO. The relationship between eNO levels and the percentage of eosinophils in peripheral blood in our study is compatible to a recent study.[12] The correlation between eNO levels and eosinophilia in sputum is not clearly demonstrated in our study. In our study, samples were collected from only 46 subjects (7 of 35 subjects in the ICS (+) group or from 39 of 62 subjects in the ICS (–) group), because the samples were collected from spontaneously expectorated sputum. The number of samples was too small to divide the subjects into two groups for analysis. Recent studies have shown that eNO levels correlate with eosinophils in induced sputum in steroid-naïve patients, but not in asthma patients treated with ICS.[12,19] Our sputum results are mainly reflected in the steroid-naïve subjects. These findings suggest that eNO is a useful marker of eosinophilic airway inflammation in patients without ICS treatment, and eNO partially reflects eosinophilic inflammation in those with ICS treatment.

In asthma patients with airflow limitation who were

treated either with or without ICS, eNO levels were significantly associated with ΔFEV_1. The data from the asthma patients with airflow limitation and those treated with ICS suggest that eNO levels may be useful for detecting deterioration of asthma control, and the findings from the asthma patients treated without ICS indicate that eNO may be useful for diagnosing asthma in the patients with reversible airway obstruction. In a recent study, Sippel *et al*. noted that eNO levels correlate with reversibility of airflow obstruction in asthma patients treated with or without ICS and that elevated eNO levels may reflect the presence of inflammation treatable with anti-inflammatory drugs.[20] Our current findings support their conclusion.

In asthma patients with normal respiratory function who were treated either with or without ICS, there was no significant relationship between eNO and ΔFEV_1. In this group, eNO levels in some subjects were high despite low levels of ΔFEV_1. In such subjects (who have 'cough variant' asthma), the diagnosis of asthma is often difficult because lung function tests such as spirometry or the reversibility of airway obstruction have a low sensitivity for diagnosing asthma in those patients, so that the markers of airway inflammation can be useful in such subjects. The tests for bronchial hyperresponsiveness and eosinophil count in induced sputum have high sensitivity, but they are relatively invasive.[27] eNO measurement offers advantages over the previous tests because it is quick, easy to perform, and absolutely noninvasive. Previous studies have shown that eNO is useful in diagnosing mild asthma,[17,18] and our results are compatible with the findings in these studies. In addition, our findings for asthma patients treated without ICS show that eNO measurement may be more useful for diagnosing asthma than the test of reversible airway obstruction. Furthermore, our data from subjects treated with ICS show that the eNO may be useful in determining which patients who would benefit from anti-inflammatory therapy among asthma patients with normal pulmonary function. In fact, recent studies[4,20] have shown that eNO levels are correlated with various measures of disease control in asthma, and that eNO measurement is useful for assessing the treatment effect in asthma patients. Our data are compatible with these studies.

In summary, the current study shows that the eNO levels measured using an off-line method correlate with the degree of reversible obstructive impairment in Japanese adult asthma patients with airflow limitation. Our findings suggest that monitoring eNO will facilitate the diagnosis and management of bronchial asthma. We need further long-term studies and studies to find the association between eNO levels and bronchial hyperresponsiveness to determine the clinical value of monitoring eNO levels.

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