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ORIGINAL ARTICLE

Relationship between Exhaled Nitric Oxide Measured by Two Offline Methods and Bronchial Hyperresponsiveness in Japanese Adults with Asthma

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ABSTRACT

Background: Exhaled nitric oxide (eNO) is a useful marker of eosinophilic airway inflammation in asthmatics. There have been no studies to show the relationship between eNO measured by offline methods and the degree of bronchial hyperresponsiveness in asthmatic patients treated with inhaled corticosteroids.

Methods: The study population comprised asthmatics at our outpatient clinic. We measured eNO levels by two methods ("eNOs" was measured with a Sievers kit; and "eNOc" was measured with a kit from the Center for Environmental Information Science, Japan). We also used spirometry to test bronchial hyperresponsiveness to acetylcholine (PC_{20Ach}).

Results: We recruited 192 stable asthmatics. There was a significant relationship between eNOs and eNOc ($r = 0.919$, $p < 0.001$). $LogPC_{20Ach}$ levels were negatively correlated with eNOs or eNOc levels (eNOs, $r = -0.31$, $p < 0.001$; eNOc, $r = -0.23$, $p = 0.0013$). We classified the subjects into two groups based on eNOs levels ((A) the subjects with high eNOs levels ($n = 92$) and (B) the subjects with normal eNOs levels ($n = 100$)) ; $logPC_{20Ach}$ was significantly correlated with eNOs ($r = -0.34$, $p = 0.001$) or eNOc ($r = -0.28$, $p = 0.0075$) but not correlated with $\%FEV_1$ in (A), whereas $logPC_{20Ach}$ was not significantly correlated with eNO but significantly correlated with $\%FEV_1$ ($r = 0.33$, $p = 0.002$) in (B).

Conclusions: Levels of eNOs and eNOc were correlated with the degree of bronchial hyperresponsiveness to acetylcholine in adult asthmatics treated with inhaled corticosteroids. Our findings suggest that offline monitoring of eNO will facilitate the management of bronchial asthma in patients treated with these drugs.

KEY WORDS

asthma, bronchial hyperresponsiveness, exhaled nitric oxide, offline method

ABBREVIATIONS

eNO, exhaled nitric oxide; BHR, bronchial hyperresponsiveness; CEIS, The Center for Environmental Information Science (Tokyo, Japan); PC_{20Ach}, provocative concentration of acetylcholine in inhaled aerosols that leads to a 20% fall in FEV_1.

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INTRODUCTION

Bronchial asthma is characterized by eosinophilic bronchial inflammation. Inhaled corticosteroids (ICS), the mainstay of asthma treatment, are effective because they prevent this inflammatory process. Therefore, the quantification of airway inflammation may provide additional information for both the diagnosis and management of bronchial asthma. However, the current asthma management guideline (GINA: Global Initiative for Asthma) relies on the monitoring of respiratory function and symptoms.[1] Three studies have demonstrated that the addition of alternative monitoring markers, such as bronchial hyperresponsiveness (BHR), eosinophilia in induced sputum, and exhaled nitric oxide (eNO) concentration, to the current guidelines on dose adjustment of ICS leads to improved outcomes.[2-4]

According to the recent studies, eNO is a useful marker of airway eosinophilic inflammation in asthma.[5,6] The increased levels of eNO are due to the 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 asthmatics than in healthy subjects,[10] and eNO levels fall after treatment with corticosteroids.[11] In asthmatics treated with ICS, eNO levels are correlated with the following markers of disease control: asthma symptoms within the past 2 weeks, disease score, and reversibility of airflow obstruction.[12] eNO is useful in the clinical management of asthmatic patients treated with ICS.[4]

However, despite the utility of eNO measurement, NO analyzers are too expensive for widespread use by general practitioners, for whom the offline (bag collection) method of eNO measurement may be more affordable.[5,6,13] In our previous study, eNO measurements taken with a Sievers (Sievers Instruments Division, Ionics, Inc., Boulder, CO) bag collection kit were significantly correlated with the level of bronchial reversibility and airway eosinophilic inflammation both in patients on ICS and not on ICS.[14] Also, recent studies have shown that the results obtained with a new offline method developed by the Center for Environmental Information Science (CEIS) in Tokyo is equivalent to those with the online method.[15,16] We sought to investigate the relationship between the eNO levels measured by these two offline methods and the level of BHR in Japanese asthmatic patients.

METHODS

SUBJECTS

The study population was recruited from adult outpatients with bronchial asthma (n = 192) at the Clinic of Allergy and Respiratory Medicine, Sagamihara National Hospital, between June 2003 and April 2005. All patients gave full informed consent to participate in the study. Each subject underwent a standard clinical assessment, which included history, physical examination, and chest radiography. The diagnosis of asthma was based on application of the GINA guideline performed by an experienced respiratory physician blinded to the results of eNO measurement.[1] Therapy was chosen in accordance with this guideline.[1] Atopy was indicated by a positive skin test to mites or house dust or by serum IgE >250 IU/mL. Exclusion criteria included current smokers or ex-smokers of >20 pack-years, rescue use of oral corticosteroids within the preceding 4 weeks, pregnancy, and any other respiratory disease. The study protocol was approved by the Ethics Committee at our hospital (no. 14, 2003).

MEASUREMENT OF eNOs AND eNOc

Exhaled air was collected with Sievers bag collection kits in accordance with the method used previously[14,17] and by the CEIS method reported by Munakata *et al*.[15] Briefly, subjects took a deep breath of room air through the NO scavenging filter and exhaled through a mouthpiece with a flow rate of 70 mL/s against an expiratory resistance of 10 cm H_2O; 5 seconds later, the exhaled air was collected into the 1.5-L Mylar bag provided in the kit. Immediately after the collection by Sievers's kit, Exhaled air was collected with the CEIS kit. Subjects took a deep breath of room air and exhaled through a mouthpiece with a flow rate of 50 mL/s against an expiratory resistance of 15 cm H_2O; 5 seconds later, exhaled air was collected into the 1.5-L Mylar bag provided in the kit. The NO concentration in the collected exhaled air was stored at room temperature and measured within 12 hours. The air was drawn out of the balloons at 200 mL/min into an NO chemiluminescence analyzer (NOA model 280A, Sievers Instruments) with a response time of 200 milliseconds. Measurements of eNO were labeled "eNOs" (taken with the Sievers kit) and "eNOc" (taken with the CEIS kit). On the basis of recent studies, the levels of eNOs are about 80% of those with online methods[14,17] and the levels of eNOc are equivalent to those with online methods.[15]

MEASUREMENT OF BHR TO ACETYLCHOLINE

Inhalation testing was performed by a modification of the method described by Chai *et al*.[18] Acetylcholine chloride (Ach; Ovisort; Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) at concentrations of 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10, and 20 mg/mL was prepared by dilution in buffered saline solution (pH 7.4). After the exhaled air samples for eNO measurement had been collected, all subjects underwent spirometry with an electric spirometer (Minato Autospiro AS-303; Minato Medical Science Co., Ltd., Osaka, Japan). Subjects inhaled Ach aerosol from a nebulizer (DeVilbiss 646; DeVilbiss Co., Somerset, PA) by tidal breathing for 2 minutes. The operating airflow rate of this device was

Table 1 Patient demographics

No.	192
Sex (M/F)	59/133
Age	48.9±16.6
Atopy/non-atopy	125/67
Duration of ICS therapy (year)	7.69±8.66
The dose of ICS (mcg/day, beclomethasone equivalent)	963±705
FEV_1 (L)	2.32±0.71
$\%FEV_1$ (% of predicted)	88.6±15.6
%MMF (% of predicted)	66.0±22.8
$\%V_{50}$ (% of predicted)	67.6±25.0
$LogPC_{20Ach}$	3.80±0.72
Eosinophils in peripheral blood (%)	4.0 (2.9－5.1)
$eNO_{SIEVERS}$ (ppb)	25.5 (20.8－30.2)
eNO_{CEIS} (ppb)	34.3 (28.0－40.6)

The data are presented as mean ± SD, or as median (the range of 95%CI).

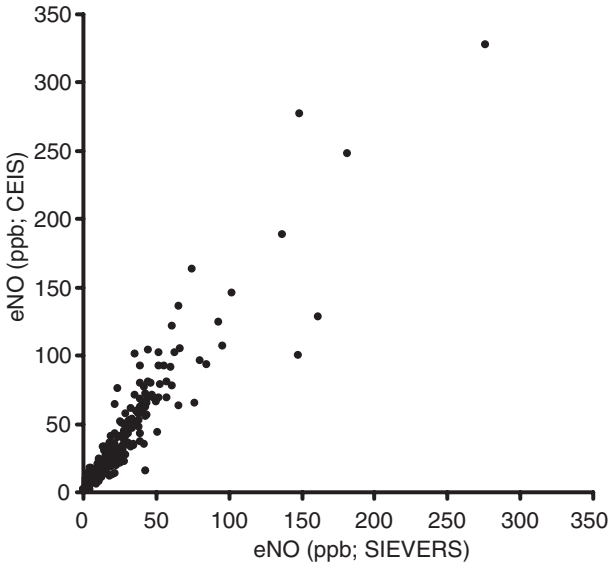

Fig. 1 Correlation between eNOs and eNOc ($r = 0.919$, $p < 0.001$).

5 L/min. Subjects inhaled the Ach aerosol at increasing concentrations until the FEV_1 fell by >20% of its baseline value or until maximum concentration. Bronchial sensitivity was expressed as PC_{20Ach} and was defined as the provocative concentration of the agonist in the inhaled aerosol leading to a fall in FEV_1 of 20%. PC_{20Ach} was estimated by linear interpolation.

STATISTICAL ANALYSIS

Data are shown as means ± SD, but shown as median and their 95% confidence interval (CI) for eosinophils in peripheral blood, eNOs and eNOc. Correlations were determined by the Spearman rank correlation. A paired sample t test was used to analyze parameters between the group with high eNOs and that with normal eNOs in Table 2. $p < 0.05$ was considered significant.

RESULTS

We recruited 192 stable asthmatics for the study (Table 1). All subjects were treated with ICS and were asymptomatic. There was a significant correlation between eNOs and eNOc ($r = 0.919$, $p < 0.001$, Fig. 1). There was a weakly significant correlation between $\%FEV_1$ and eNOs or eNOc (eNOs, $r = -0.19$, $p = 0.011$, Fig. 2A; eNOc, $r = -0.19$, $p = 0.012$, Fig. 2B). $LogPC_{20Ach}$ was significantly negatively correlated with eNOs or eNOc (eNOs, $r = -0.31$, $p < 0.001$, Fig. 3A; eNOc, $r = -0.23$, $p = 0.0013$, Fig. 3B). In the 71 subjects who consented to the collection of peripheral blood samples, the percentage of eosinophils in the blood was significantly correlated with eNOs or eNOc (eNOs, $r = 0.40$, $p = 0.0018$, Fig. 4A; eNOc, $r = 0.37$, $p = 0.0017$, Fig. 4B).

In the previous study,[21] there was no relationship between BHR and eNO in well-controlled asthmatics treated with ICS. For the purpose of comparing the previous study and our study, we classified the subjects into two groups based on the level of eNOs; (A) the group with high eNOs levels (eNOs ≧ 27.2 ppb) and (B) that with normal eNOs levels (eNOs < 27.2 ppb). The normal range of eNOs was determined in 14 healthy, non-atopic, non-smoking volunteers (median 21.1 ppb, 95%CI was 15.0–27.1 ppb). As shown in Table 2, $\%FEV_1$, %MMF, $\%V_{50}$, $logPC_{20}$ was significantly decreased and eosinophils in the peripheral blood were significantly increased in (A), compared with (B). Moreover, as shown in Table 3, there was a significant relationship between $logPC_{20Ach}$ and eNOs, eNOc, or eosinophils in blood, but no significant relationship between $logPC_{20Ach}$ and $\%FEV_1$, %MMF or $\%V_{50}$ in (A). On the contrary, in (B) group, there was a significant relationship between $logPC_{20Ach}$ and $\%FEV_1$, %MMF or $\%V_{50}$, but no significant relationship between $logPC_{20Ach}$ and eNOs, eNOc, or eosinophils in blood.

DISCUSSION

In asthmatics treated with ICS, eNOs and eNOc were significantly negatively associated with $logPC_{20Ach}$.

There has been controversy over the relationship between eNO and BHR levels in steroid-treated asthmatics. In over 8000 steroid-naïve adolescents in Norway, eNO levels were significantly related to BHR levels.[19] Several studies have shown that, in steroid-naïve asthmatics, eNO levels were correlated with the degree of BHR in response to methacholine or histamine, but there was no such correlation in steroid-treated asthmatics.[20-22] However, in contrast, Reid and coworkers showed that, in steroid-treated asth-

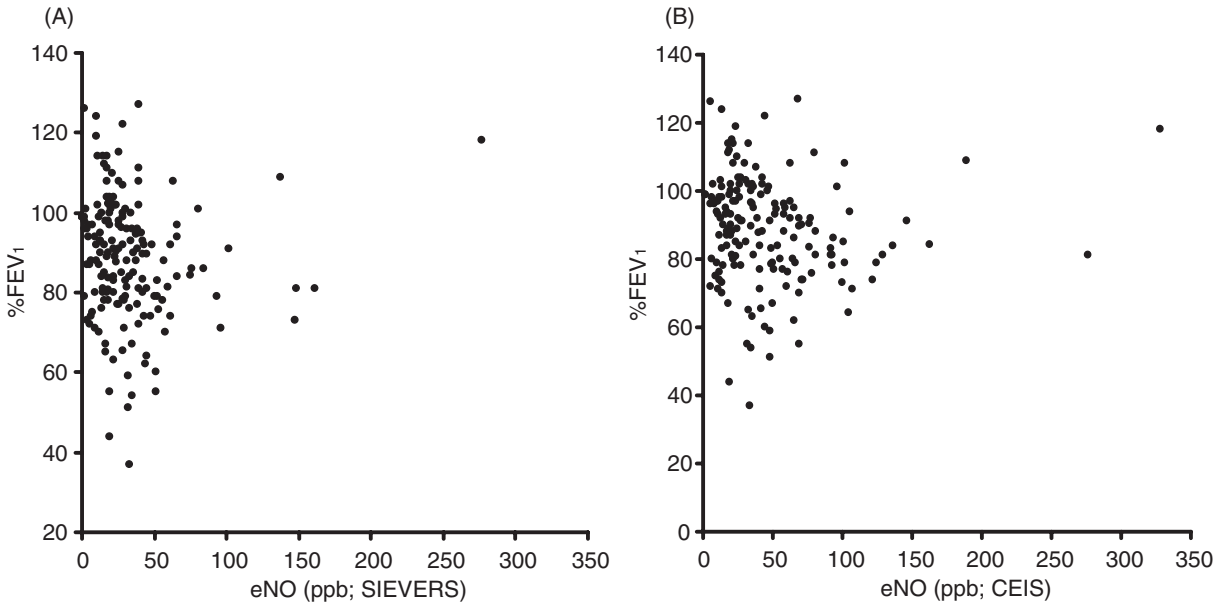

Fig. 2 Correlation between eNO and %FEV_1. (**A**) eNOs ($r = -0.19$, $p = 0.011$) and (**B**) eNOc ($r = -0.19$, $p = 0.012$).

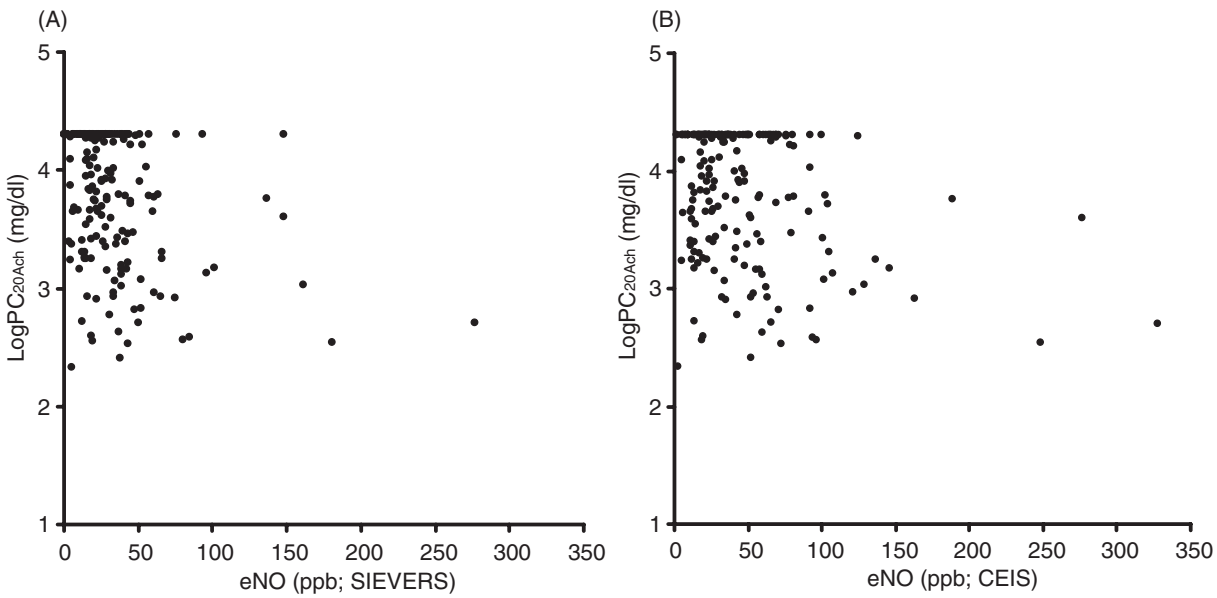

Fig. 3 Correlation between eNO and $logPC_{20Ach}$. (**A**) eNOs ($r = -0.31$, $p < 0.001$) and (**B**) eNOc ($r = -0.23$, $p = 0.0013$).

matics, eNO levels were correlated with the degree of BHR to methacholine.[23]

There are two possible explanations for these contradictory results: one is the difference between eNO and BHR in terms of their response to ICS therapy, and the other is the difference in the types of subjects recruited in each of these previous studies. The eNO level reflects airway eosinophilic inflammation. eNO level and the percentage of eosinophils in the peripheral blood were significantly correlated, and their relationship was comparable to that in a recent study.[14,23] These findings suggest that eNO is a useful marker of eosinophilic airway inflammation in patients treated with ICS. Airway inflammation causes BHR, but the levels of BHR not only reflected the airway inflammation but also remodeling or lung function. Moreover, eNO behaves as a rapid response marker. In previous studies, ICS therapy decreased

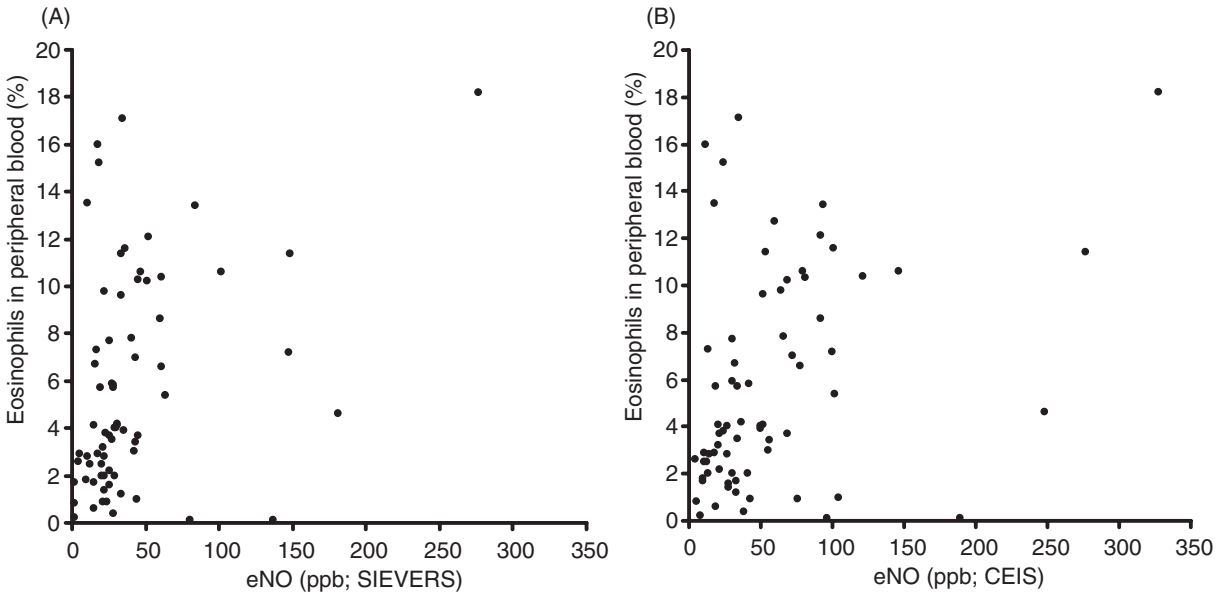

Fig. 4 Correlation between eNO and eosinophil count (%) in peripheral blood in 71 asthmatics. (**A**) eNOs ($r = 0.40$, $p = 0.0018$) and (**B**) eNOc ($r = 0.37$, $p = 0.0017$).

Table 2 Patients demographics based on the levels of eNOs

	(A) $eNO_{SIEVERS} \geqq 27.2$ ppb	(B) eNOs $<$ 27.2 ppb	*p*-value
No.	92	100	
Duration of ICS therapy (year)	6.72±7.39	8.58±9.63	0.13
The dose of ICS (mcg/day, beclomethasone equivalent)	923±617	1000±777	0.45
$\%FEV_1$ (% of predicted)	85.3±15.7	91.7±14.8	0.007
%MMF (% of predicted)	63.1±21.8	68.6±23.5	0.09
$\%V_{50}$ (% of predicted)	65.4±24.8	69.5±25.1	0.26
$LogPC_{20Ach}$	3.66±0.59	3.93±0.48	0.001
Eosinophils in peripheral blood (%)	5.9 (4.4－7.4)	5.0 (3.1－6.9)	0.009
$eNO_{SIEVERS}$ (ppb)	40.1 (32.2－48.0)	16.0 (14.6－17.4)	
eNO_{CEIS} (ppb)	60.2 (49.9－70.5)	20.2 (17.8－22.7)	

The data are presented as mean ± SD, or as median (the range of 95%CI).

27.1 ppb was the limited value of normal range in $eNO_{SIEVERS}$.

The range was determined in 14 healthy, non-atopic, non-smoking volunteers (median 21.1 ppb, 95%CI was 15.0－27.1 ppb).

Table 3 Relationship between $logPC_{20Ach}$ and other parameters

	(A) $eNO_{SIEVERS} \geqq 27.2$ ppb		(B) eNOs $<$ 27.2 ppb	
	r	*p*-value	*r*	*p*-value
$\%FEV_1$ (% of predicted)	0.028	0.79	0.33	0.002
%MMF (% of predicted)	0.18	0.08	0.48	$<$ 0.001
$\%V_{50}$ (% of predicted)	0.16	0.11	0.49	$<$ 0.001
Eosinophils in peripheral blood (%)	− 0.37	0.021	0.13	0.468
$eNO_{SIEVERS}$ (ppb)	− 0.34	0.001	− 0.12	0.211
eNO_{CEIS} (ppb)	− 0.28	0.0075	− 0.04	0.662

r: Pearson correlation coefficient between PC_{20Ach} and other parameters.

the eNO level within 4 weeks, whereas BHR took 8 weeks or more to respond to ICS.[24] These findings suggest that eNO and BHR were significantly correlated, but we should pay attention to the difference of these two factors.

In previous studies in which eNO levels were not correlated with BHR in ICS-treated subjects,[20,21] the subjects were well-controlled with ICS (asymptomatic and with almost normal function and normal eNO levels; a substantial proportion had negative inhalational challenge results). Our subjects were asymptomatic, but about 50% of them had high eNOs levels, suggesting that our subjects included patients with only partly controlled disease (Table 2). In the subjects with normal eNOs levels in our study, there was no significant relationship between $\log PC_{20Ach}$ and eNOs or eNOc, compatible with Leuppi's study (Table 3). And also, in these subjects, there was a significant relationship between $\log PC_{20Ach}$ and parameters of the pulmonary function test. This finding suggests that BHR may reflect remodeling in asthmatics whose air inflammation was well-controlled. On the other hand, in the subjects with high levels of eNOs, $\log PC_{20Ach}$ was correlated with eNOs or eNOc, but was not correlated with $\%FEV_1$, %MMF, or $\%V_{50}$. This shows that BHR can reflect subclinical airway inflammation. These findings suggest that eNO is useful as a marker of disease control in asthmatics treated with ICS, and this is probably because eNO measurement may detect subclinical airway inflammation. Moreover, add-on of anti-inflammatory drugs may be effective in the subjects with high eNO levels, whereas add-on of bronchodilators may be effective in the subjects with normal eNO levels and obstructive impairment. The measurement of eNO can complement the pulmonary function test as a following marker of asthma. According to previous studies, eNO levels were correlated with markers such as FEV_1 or bronchial hyperresponsiveness to saline of disease control[12,25] and eNO was considered useful in the clinical management[4] of ICS-treated asthmatic patients. Reid's study and Smith's study support our conclusion. However, further prospective studies are needed to explain this hypothesis.

Previous studies on the relationship between BHR and eNO were based on the online methods. In this study, the subjects were not measured with the online method, but the levels of eNOc were almost equivalent to those with online method.[14] The CEIS's method can be more useful due to this point, but because there are only few reports previously, we need further studies based on CEIS's method.

In summary, the eNO levels measured by the two offline methods were significantly correlated with the degree of BHR to acetylcholine in adult asthmatics treated with ICS. Our findings suggest that monitoring of NO will facilitate management of bronchial asthma in ICS-treated patients, but we need further, long-term studies to determine the clinical value of following eNO levels in these patients.

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REFERENCES

1. National Institudes of Health, National Heart, Lung, and Blood Institute. *Global Strategy for Asthma Management and Preventation: Global Initiative For Asthma 2002.* NIH Publication No 02-3659. Bethesda: National Institute of Health, 2002.
2. Sont JK, Willems LN, Bel EH, Van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. *Am J Respir Crit Care Med* 1999; **159**: 1043-51.
3. Green RH, Brightling CE, McKenna S *et al*. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;**360**:1715-21.
4. Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. *N Engl J Med* 2005;**352**: 2163-73.
5. American Thoracic Society Board of Directors. Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 1999;**160**:2104-17.
6. American Thoracic Society Board of Directors. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;**171**:912-30.
7. Van den Toorn LM, Overbeek SE, De Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. *Am J Respir Crit Care Med* 2001;**164**:2107-13.
8. Hamid Q, Springall DR, Riveros-Moreno V *et al*. Induction of nitric oxide synthase in asthma. *Lancet* 1993;**342**:1510-3.
9. Saleh D, Ernst P, Lim S, Barnes PJ, Giaid A. Increased formation of the potent oxidant peroxynitrite in the airways of asthmatic patients is associated with induction of nitric oxide synthase: effect of inhaled glucocorticoid. *Faseb J* 1998;**12**:929-37.
10. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994;**343**:133-5.
11. Yates DH, Kharitonov SA, Robbins RA, Thomas PS, Barnes PJ. Effect of a nitric oxide synthase inhibitor and a glucocorticosteroid on exhaled nitric oxide. *Am J Respir Crit Care Med* 1995;**152**:892-6.
12. Sippel JM, Holden WE, Tilles SA *et al*. Exhaled nitric oxide levels correlate with measures of disease control in asthma. *J Allergy Clin Immunol* 2000;**106**:645-50.
13. Silkoff PE, Stevens A, Pak J, Bucher-Bartelson B, Martin

RJ. A method for the standardized offline collection of exhaled nitric oxide. *Chest* 1999;**116**:754-9.
14. Tsuburai T, Tsurikisawa N, Taniguchi M *et al*. The relationship between exhaled nitric oxide measured with an off-line method and airway reversible obstruction in Japanese adults with asthma. *Allergol Int* 2007;**56**:37-43.
15. Munakata M, Saito J, Hasunuma H, Ishimaru Y, Kanegae H, Kudo S. Development of new method for off-line measurement of exhaled NO (FENO) compatible to on-line method. *Eur Respir J* 2004;**24**:267s.
16. Murata A, Kida K, Hasunuma H *et al*. Environmental influence on the measurement of exhaled nitric oxide concentration in school children: special reference to methodology. *J Nippon Med Sch* 2007;**74**:30-6.
17. Tsuburai T, Suzuki S, Tsurikisawa N *et al*. [The methodological aspects of off-line sampling of exhaled air for nitric oxide measurement in Japanese]. *Nihon Kokyuki Gakkai Zasshi* 2007;**45**:160-5(in Japanese).
18. Chai H, Farr RS, Froehlich LA *et al*. Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975;**56**:323-7.
19. Henriksen AH, Lingaas-Holmen T, Sue-Chu M, Bjermer L. Combined use of exhaled nitric oxide and airway hyperresponsiveness in characterizing asthma in a large population survey. *Eur Respir J* 2000;**15**:849-55.
20. Ichinose M, Takahashi T, Sugiura H *et al*. Baseline airway hyperresponsiveness and its reversible component: role of airway inflammation and airway calibre. *Eur Respir J* 2000;**15**:248-53.
21. Leuppi JD, Salome CM, Jenkins CR *et al*. Markers of airway inflammation and airway hyperresponsiveness in patients with well-controlled asthma. *Eur Respir J* 2001;**18**: 444-50.
22. Nogami H, Shoji S, Nishima S. Exhaled nitric oxide as a simple assessment of airway hyperresponsiveness in bronchial asthma and chronic cough patients. *J Asthma* 2003;**40**:653-9.
23. Reid DW, Johns DP, Feltis B, Ward C, Walters EH. Exhaled nitric oxide continues to reflect airway hyperresponsiveness and disease activity in inhaled corticosteroid-treated adult asthmatic patients. *Respirology* 2003;**8**: 479-86.
24. Fujimoto K, Yamaguchi S, Urushibata K *et al*. Characteristics of asthma resistant to moderate dose inhaled corticosteroid treatment on bronchial hyperresponsiveness. *Intern Med* 2006;**45**:843-9.
25. Jones SL, Kittelson J, Cowan JO *et al*. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. *Am J Respir Crit Care Med* 2001;**164**:738-43.