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Difference between two exhaled nitric oxide analyzers, NIOX VERO[®] electrochemical hand-held analyzer and NOA280i[®] chemiluminescence stationary analyzer

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ABSTRACT

Background: Fractional exhaled nitric oxide (FE_{NO}) is useful for the evaluation of eosinophilic airway inflammation, including that seen in asthma. Although a new electrochemical hand-held FE_{NO} analyzer, the NIOX VERO[®] (Aerocrine AB, Solna, Sweden), is clinically convenient to use, it has not been fully compared with the chemiluminescence stationary electrochemical analyzer NOA280i[®] (Sievers Instruments, Boulder, CO, USA) in terms of the level of measured FE_{NO}. The aim of this study was to determine whether there is a difference between the two analyzers. **Methods:** The FE_{NO} levels measured with both NIOX VERO[®] and NOA280i[®] were evaluated in 1,369 adults at Juntendo University Hospital from May 2016 to October 2016. **Results:** The median FE_{NO} level measured with the NIOX VERO[®] was significantly lower than that measured with the NOA280i[®] (41 ppb, range 5–368 ppb vs. 29 ppb, range 5–251 ppb; $p < 0.001$). There was a strong positive correlation in the measurement of FE_{NO} level between the NOA280i[®] and the NIOX VERO[®] ($r = 0.942$, $p < 0.001$). The following conversion equation was calculated: $FE_{NO} (NOA280i) = 1.362 (SE, 0.661) + 1.384 (SE, 0.021) \times FE_{NO} (NIOX VERO)$. **Conclusions:** To our best knowledge, we have provided the first report showing that the measured FE_{NO} level with the NIOX VERO[®] was approximately 30% lower than that with the NOA280i[®] and that there was a significant correlation between the measurements of these two devices. The correction equation that we provided may help assess the data obtained by these two analyzers.

Abbreviations: ATS: American Thoracic Society; BMI: Body mass index; ERS: European Respiratory Society; FE_{NO}: Fractional exhaled nitric oxide; GINA: Global Initiative for Asthma; NO: Nitric oxide; ppb: Parts per billion; ROC: Receiver operating characteristic; SD: Standard deviation

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Asthma; fractional exhaled nitric oxide; NIOX VERO[®]; nitric oxide analyzer; NOA280i[®]

Introduction

Fractional exhaled nitric oxide (FE_{NO}) level is a quantitative, noninvasive, simple, and safe marker of eosinophilic airway inflammation in patients with several respiratory diseases, including bronchial asthma [1]. Its measurement has been a useful method to predict the existence of those diseases, to guide treatment, and to assess the response to anti-inflammatory therapies [2]. Although the importance of FE_{NO} measurement has increased, it

has been difficult to interpret the measurements because the available nitric oxide (NO) analyzers have been reported to have differences in FE_{NO} levels [3–6].

Interpretation of FE_{NO} measurements should be based on the official American Thoracic Society (ATS) clinical practice guidelines. The standard cut-off values of FE_{NO} were established mainly with the use of chemiluminescence stationary NO analyzers, one of which was Sievers NOA280i[®] (GE Analytical

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Instruments, Boulder, CO, USA) [1]. On the other hand, a new electrochemical hand-held NO analyzer, the NIOX VERO® (Aerocrine AB, Solna, Sweden), was developed instead of NIOX MINO® (Aerocrine AB, Solna, Sweden). In Japan, these electrochemical hand-held analyzers are used often because they are portable, highly versatile, and covered by the national health insurance.

Although there have been several studies that compared the FE_{NO} levels measured with the NIOX MINO® and those measured with other analyzers, the NIOX VERO® has not been sufficiently evaluated and compared with the other analyzers. The aim of this study was to confirm the correlation of FE_{NO} levels between the NIOX VERO® electrochemical hand-held and the chemiluminescence stationary NOA280i® NO analyzers and to calculate the conversion equation for the FE_{NO} levels measured with the two analyzers.

Methods

Study population

Patients who visited the outpatient clinic of Juntendo University Hospital (Tokyo, Japan) from May 2016 to October 2016 for follow-up of diagnosed or suspected asthma were enrolled in the study. Asthma was diagnosed based on a clinical history of episodic symptoms with airflow limitation and on variations in pulmonary function, either by forced expiratory volume in 1 s or by peak expiratory flow, in accordance with the Global Initiative for Asthma (GINA) guidelines [5]. The present study was reviewed and approved by the Juntendo University Research Ethics Committee. This study was registered in the UMIN Clinical Trial Registry (UMIN000024887) (<http://www.umin.ac.jp/>).

Measurement of the fractional exhaled nitric oxide

The level of FE_{NO} was measured according to the manufacturer's instructions and the international recommendations of the ATS/European Respiratory Society (ERS), as described previously [2]. Measurements were performed using the NIOX VERO®, followed by the NOA280i® on the same day. Measurements using the NIOX VERO® were repeatedly performed in order to be acceptable. In the analyzer algorithm, the subjects were asked to exhale into the device at a pressure of 10–20 cm H_2O in order to keep a constant flow rate of 50 mL/s; a single acceptable level was recorded for the analysis. The FE_{NO} levels measured with the NOA 280i® were evaluated online during a single breath of at least 5 cm H_2O

for closure of the velopharyngeal aperture and to prevent unnecessary NO contamination from the nasal cavity. Subjects were instructed to inhale NO-free air and to exhale for 10 s at a flow rate of 50 mL/s. The mean of three sufficient measurements that sustained a plateau of 10 s was deemed acceptable.

Statistical analysis

Spearman's test was used to calculate the correlation coefficient for FE_{NO} levels measured with the NOA280i® and those with the NIOX VERO®. The Bland–Altman method was used to evaluate the agreement between the two devices. Comparison of the FE_{NO} levels measured with the two devices was performed by Wilcoxon signed rank test. The conversion equation was calculated using linear regression with robust standard errors. Differences were deemed statistically significant when the p values were 0.05 or less. Receiver operating characteristic (ROC) curve analyses for FE_{NO} levels were performed to discriminate the patients diagnosed with asthma from the patients with other unconfirmed diagnoses. The optimum cut-off value was determined with Youden's index criteria. Statistical analysis was performed using the software package STATA/SE 14.2 (Stata Corp. LP, TX, USA).

Results

The demographic and clinical characteristics of the 1,369 subjects evaluated in this study are shown in Table 1. The median age was 57 years (range, 15–90 years) and there were 752 (54.9%) women. There were 756 (55.2%) patients diagnosed with asthma, whereas the remaining 613 (44.8%) had other unconfirmed diagnoses for prolonged cough or chronic cough, including asthma, cough-variant asthma, chronic obstructive pulmonary disease, gastroesophageal reflux, and post-infectious cough.

All the enrolled subjects were able to perform acceptable FE_{NO} measurements using both devices. The median FE_{NO} level was 41 parts per billion (ppb) (range, 5–368 ppb) for NOA 280i® and 29 ppb (range, 5–251 ppb) for NIOX VERO® (Table 1). There was a strong positive correlation in the FE_{NO} levels between the NOA280i® and the NIOX VERO® ($r = 0.942$, $p < 0.001$) (Figure 1). The FE_{NO} levels were significantly lower with the NIOX VERO® than with the NOA280i®, even among patients with asthma, those with other unconfirmed diagnoses, and those who were current smokers (Figure 2). Moreover, the FE_{NO}

Table 1. Baseline characteristics of the study population.

Variable	Total <i>n</i> = 1369	Diagnosed as Asthma <i>n</i> = 756	Unconfirmed Diagnosis <i>n</i> = 613	<i>p</i> -value
Age (y), median (range)	57 (15–90)	56 (15–90)	59 (15–88)	0.098
Male, <i>n</i> (%)	617 (45.1)	332 (43.9)	285 (46.5)	0.353
Height (cm)	161.9 ± 8.8	161.7 ± 9.0	162.0 ± 8.6	0.650
Weight (kg)	61.0 ± 12.9	62.3 ± 13.4	59.4 ± 12.1	<0.001*
BMI (kg/m ²)	23.2 ± 4.1	23.7 ± 4.3	22.5 ± 3.7	<0.001*
Never smoker, <i>n</i> (%)	795 (58.1)	460 (60.8)	335 (54.6)	0.024*
Current smoker, <i>n</i> (%)	119 (8.7)	57 (7.5)	62 (10.1)	0.101
Asthma, <i>n</i> (%)	756 (55.2)	756 (100)	0 (0)	
FE _{NO} (ppb), median (range)				
NIOX VERO [®]	29 (5–251)	33 (5–251)	26 (6–242)	<0.001*
NOA280i [®]	41.0 (5.1–368)	46.2 (5.1–368)	35.1 (5.7–298)	<0.001*

Note. Data are presented as mean ± SD, unless otherwise indicated.

BMI: body mass index; SD: standard deviation.

*Indicates *p* < 0.05, Diagnosed as asthma versus unconfirmed diagnosis.

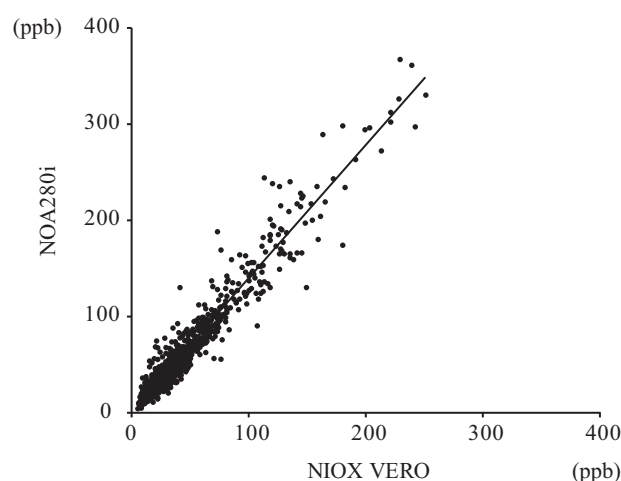


Figure 1. Linear regression analysis between the NOA280i[®] and the NIOX VERO[®]. The coefficient of determination of the linear regression was 0.9367 ($R^2 = 0.9367$). There was a positive correlation between the FE_{NO} levels measured with NOA280i[®] and those measured with the NIOX VERO[®] ($r = 0.942$, $p < 0.001$). FE_{NO}, fractional exhaled nitric oxide; ppb, parts per billion.

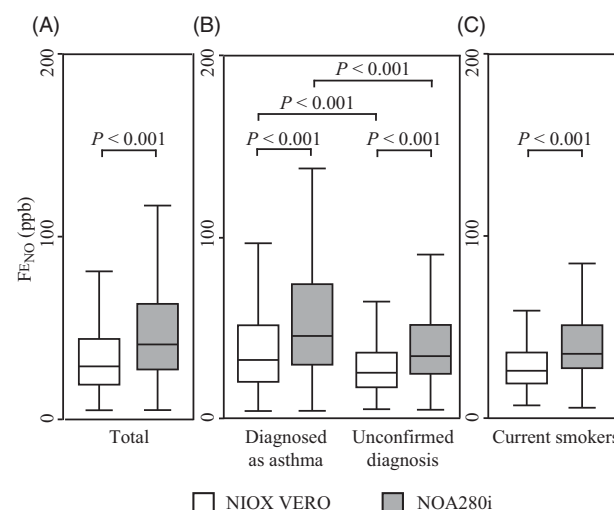


Figure 2. Comparison between the two analyzers. The FE_{NO} levels measured with NIOX VERO[®] were significantly lower than those measured with the NOA280i[®] in all patients (A), in patients with diagnosed asthma or with unconfirmed diagnosis (B), and patients who were current smokers (C). FE_{NO}, fractional exhaled nitric oxide; ppb, parts per billion.

levels measured by both analyzers were significantly higher in the patients diagnosed as asthma than in the patients with other unconfirmed diagnoses (Table 1 and Figure 2B).

The following equation was used to convert the derived results of the FE_{NO} level measured with the NIOX VERO[®] to that measured with the NOA 280i[®]:

$$\begin{aligned} \text{FE}_{\text{NO}}(\text{NOA280i}^{\text{®}}) = & 1.362(\text{SE}, 0.661) \\ & + 1.384(\text{SE}, 0.021) \\ & \times \text{FE}_{\text{NO}}(\text{NIOXVERO}^{\text{®}}) \end{aligned}$$

The coefficient of determination of the linear regression was 0.9367 ($R^2 = 0.9367$). The residuals, which were evenly distributed around zero, showed

no systematic pattern through the range of values of fitted NOA280i[®] and confirmed the linearity and homoscedasticity of the fit of the regression equation (Supplementary Figure S1).

The Bland–Altman plot demonstrated a proportional error between the two analyzers (Figure 3A). The difference in measurement between the two analyzers increased with increasing FE_{NO} levels (Figure 3A). However, there was no indication of a significant proportional error after the conversion equation was applied for correction of FE_{NO} levels measured with the NIOX VERO[®] (Figure 3B). These findings suggested that although most of the technical parameters of the two analyzers were similar, the correction equation calculated in this study may assist in

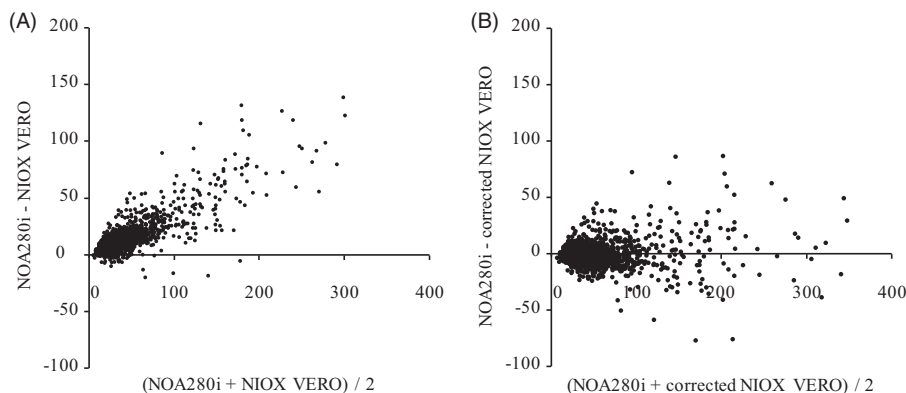


Figure 3. A Bland–Altman plot for evaluation the agreement between the two analyzers. The mean FE_{NO} values obtained with the two analyzers were plotted on the x-axis and the difference in FE_{NO} levels ($NOA280i^{\text{®}}$ – $NIOX\ VERO^{\text{®}}$) were plotted on the y-axis (A). After the applying the conversion equation to correct the levels of FE_{NO} measured with the $NIOX\ VERO^{\text{®}}$, the mean FE_{NO} value obtained with the $NOA280i^{\text{®}}$ and the corrected FE_{NO} measured with the $NIOX\ VERO^{\text{®}}$ were plotted on the x-axis; the difference in the FE_{NO} levels ($NOA280i^{\text{®}}$ – corrected $NIOX\ VERO^{\text{®}}$) were plotted on the y-axis (B). FE_{NO} , fractional exhaled nitric oxide.

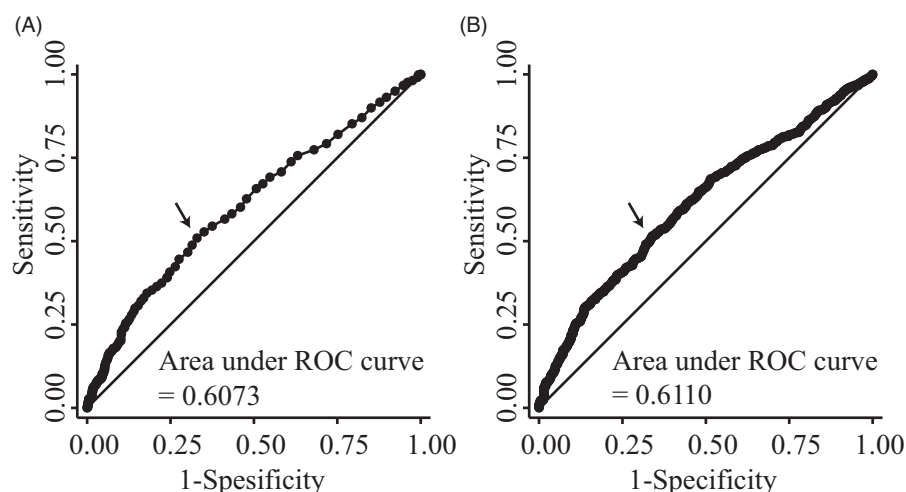


Figure 4. Receiver operating characteristic curve for FE_{NO} levels to discriminate patients diagnosed as asthma from patients with other unconfirmed diagnoses. Using Youden's index, the cut-off value was presented with an arrow. The cut-off values of FE_{NO} levels measured with the $NIOX\ VERO^{\text{®}}$ (A) and the $NOA280i^{\text{®}}$ (B) are 33 ppb (sensitivity 50.9%, specificity 67.1%) and 45.4 ppb (sensitivity 51.6%, specificity 66.1%), respectively. FE_{NO} , fractional exhaled nitric oxide; ppb, parts per billion.

the interpretation of the FE_{NO} levels measured with the $NIOX\ VERO^{\text{®}}$.

Finally, we evaluated whether the two analyzers had any difference in the discriminatory power between patients with asthma and those without asthma. ROC curve analysis was used to determine the optimal cut-off value of FE_{NO} level that would discriminate patients diagnosed with asthma from the patients with other unconfirmed diagnoses (Figure 4). The best cut-off value of FE_{NO} level was 45.4 ppb for $NOA280i^{\text{®}}$ and 33 ppb for $NIOX\ VERO^{\text{®}}$. When the best cut-off value for the FE_{NO} level from the $NIOX\ VERO^{\text{®}}$ analyzer is substituted into the above-mentioned conversion equation, the obtained value is close to that measured by the $NOA280i^{\text{®}}$.

Discussion

In this study, the FE_{NO} levels obtained with the two NO analyzers demonstrated a strong positive correlation in patients with asthma and in those with other diseases. However, the FE_{NO} levels measured with the $NIOX\ VERO^{\text{®}}$ were approximately 30% lower than those measured with the $NOA280i^{\text{®}}$. The calculated conversion equation showed no proportional error when applied to the measurements of both NO analyzers. Previous studies showed that the FE_{NO} levels obtained with the $NIOX\ MINO^{\text{®}}$ were significantly lower than those obtained with the chemiluminescence stationary analyzers $NIOX^{\text{®}}$ (Aerocrine AB, Solna, Sweden) and $NOA280i^{\text{®}}$ [6,7]. Another study indicated that the FE_{NO} levels measured with the

NIOX VERO[®] were clinically comparable to those measured with the NIOX MINO[®] [8]. Our findings suggested that the conversion equation may help in interpreting the FE_{NO} levels obtained with the NIOX VERO[®].

The NIOX VERO[®] is a battery-powered analyzer featuring longer operational and test volume lives than the NIOX MINO[®] analyzer. Moreover, NIOX VERO[®] was developed to replace the NIOX MINO[®] and enable measurement of FE_{NO} levels that range from 5 to 300 ppb [9]. Because of its portability and usefulness, the NIOX VERO[®] was regarded as a more general and effective analyzer in both clinical practice and epidemiologic studies.

Recently, a short communication reported results that were opposite to the results of the present study. That report showed that the FE_{NO} levels measured with the NIOX VERO[®] were significantly higher than those measured with the NOA280i[®] [10]. Although the reason for this discrepancy was not clear, there were some notable differences in the study design. The major difference was the number of enrolled participants, which was much higher in the present study ($n = 1,369$) than in the previous report ($n = 107$). Moreover, the subjects in the present study were adults with diagnosed asthma or with unconfirmed diagnosis, whereas the previous report included adults with diagnosed asthma. These differences may have led to the discrepant results between the two studies.

There were some limitations to be considered in interpreting the findings of the present study. First, the enrolled subjects included patients with undiagnosed cough and did not include healthy subjects. The heterogeneity of the population and the considerably large number of subjects make our data applicable to many patients regardless of disease, sex, age, or smoking history. However, this study did not conduct detailed stratification analysis of asthmatic patients based on factors such as peripheral blood eosinophil count, inhaled corticosteroid treatment, and the GINA treatment steps, and children who were younger than 15 years old were not investigated; therefore, our data may not be applicable to some populations. Second, the difference in FE_{NO} levels between the two analyzers might be accounted for by differences in the sensor technology (chemiluminescent vs. electrochemical) and calibration between the two devices. Although the analyzers were calibrated according to the instructions of the manufacturers and the ATS/ERS recommendation protocols, some other factors, such as calibration methods and calibration gas concentrations, may have influenced the

FE_{NO} levels. Third, there was a difference in the acceptable measurements for analysis between the NOA280i[®] (i.e., mean of three measurements) and the NIOX VERO[®] (i.e., the first approved measurement). However, according to the manufacturers' instrument manual, the first approved measurement of FE_{NO} levels using the NIOX VERO[®] analyzer can be used in clinical practice. Moreover, the software of the NIOX VERO[®] analyzer automatically checks that the breathing maneuver is performed according to the ATS/ERS recommendations. Fourth, we performed the measurements with the NIOX VERO[®] before the measurements with the NOA280i[®]. The ATS/ERS guidelines recommended measuring FE_{NO} levels before performing spirometry because spirometric maneuvers have been shown to transiently reduce exhaled FE_{NO} levels [2, 9]. This recommendation suggested that the FE_{NO} levels measured with the NIOX VERO[®], which took about 30 s, might be lower than those previously measured with the NOA280i[®]. Therefore, further studies using a random order of NIOX VERO[®] and NOA280i[®] FE_{NO} level measurements are needed to confirm the reproducibility of the conversion equation.

In conclusion, to our best knowledge, we have provided the first report that showed a strong positive correlation in FE_{NO} level measurement between the NIOX VERO[®] and the NOA280i[®]. We have provided a correction equation to convert the FE_{NO} levels measured with the NIOX VERO[®] to those measured with the NOA280i[®]; this equation may help compare the measured FE_{NO} levels between the two devices in both clinical practice and epidemiologic studies.

Declaration of interest

KT received honoraria from AstraZeneca K.K., Boehringer Ingelheim Japan, Inc., Eli Lilly Japan K.K., MSD K.K., and Pfizer Inc. JO is an employee of Shino-Test Corporation. RA received honoraria from Kyorin Pharmaceutical Co., Ltd. The rest of the authors have no conflict of interest.

Funding

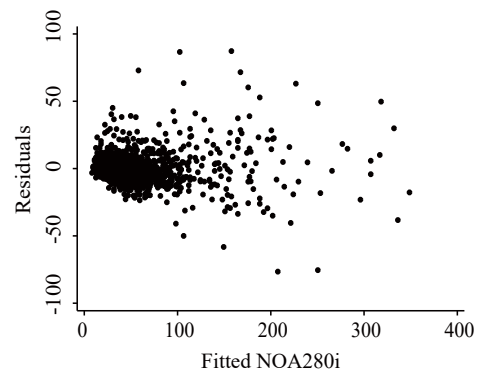
KT received a research grant from AstraZeneca K.K., Boehringer Ingelheim Japan, Inc., Chugai Pharmaceutical Co., Ltd., Eli Lilly Japan K.K., GlaxoSmithKline K.K., Kyorin Pharmaceutical Co. Ltd., MSD K.K., Ono Pharmaceutical Co., Ltd., and Taiho Pharmaceutical Co. Ltd.

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Abbreviations

ATS	American Thoracic Society
BMI	Body mass index
ERS	European Respiratory Society
FE _{NO}	Fractional exhaled nitric oxide
GINA	Global Initiative for Asthma
NO	Nitric oxide
ppb	Parts per billion
ROC	Receiver operating characteristic
SD	Standard deviation



Supplementary Figure S1. Residual plot for the linear regression between NOA280i[®] and NIOX VERO[®]. The uniformly distributed residual plot indicated assumption of normality.