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Original

Measurement of Fractional Exhaled Nitric Oxide with a Stationary Analyzer (NOA280i®) or Hand-held Analyzer (NIOX MINO®) Shows a Strong Correlation but Weaker Correlation at Higher Levels

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SUMMARY

This study investigated the difference of measured values between two types of analyzer over a wider range of fractional exhaled nitric oxide (FeNO) levels than in previous reports because some asthma patients have high FeNO levels. The equation for conversion of measured FeNO levels between the two analyzers was also determined.

Methods : Patients underwent assessment of FeNO for the diagnosis and management of asthma or for differential diagnosis of cough, FeNO levels were measured sequentially using both a stationary analyzer (NOA280i®, Sievers Inc, USA) and a hand-held analyzer (NIOX MINO®, Aerocrine Inc, Sweden).

FeNO levels were measured in a total of 167 subjects. Regression analysis of the FeNO data showed a strong positive correlation between values obtained with the two analyzers ($r=0.938$, $p<0.0001$), and the regression line was calculated as : FeNO (NOA280i®) $\times 0.63+3.79=$ FeNO (NIOX MINO®). The correlation between the two analyzers was strongest at low FeNO values and it decreased as the FeNO level increased [FeNO (NOA280i®) ≤50 ($r=0.730$, $p<0.0001$) FeNO (NOA280i®) 50 to ≤100 ($r=0.641$, $p<0.0001$), FeNO (NOA280i®) >100 to ≤150 ($r=0.607$, $p=0.0008$), and FeNO (NOA280i®) >150 ($r=0.523$, $p=0.0180$)]. Bland-Altman plot analysis between the two analyzers was 0.167 (95% confidence interval = −0.038 to 0.357, suggesting a positive difference.

Some caution is needed because the correlation between the two analyzers is weaker when the FeNO level exceeds 150.

Keywords : Fractional exhaled nitric oxide, FeNO, Asthma, NIOX MINO®, Equation for conversion

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INTRODUCTION

The fractional exhaled nitric oxide (FeNO) level has been established as a useful indicator of the state of airway inflammation in asthma patients. In patients with asthma primarily caused by eosinophils, it was reported that an increase of airway secretions, edema of the airway mucosa, and contraction of bronchial smooth muscle cause airway narrowing and elevation of the FeNO level[1]. It was also reported that measurement of FeNO is useful for the diagnosis and management of asthma and for differential diagnosis of cough, and is an essential tool for clinical management of asthma[2~4].

The conditions that should be met when FeNO measurement is performed have been specified in a guideline published by the American Thoracic Society and European Respiratory Society (ATS/ERS)[5]. A stationary analyzer (NOA280i®) has conventionally been used for measurement of FeNO. However, such analyzers have the disadvantages of being large, expensive, and require frequent maintenance, as well as not being portable. Recently, several hand-held analyzers have been developed that are useful especially in ambulatory practice, including the NIOX MINO®, NObreath®, and NIOX VERO®[6]. Several studies have already compared measured FeNO values between different analyzers and the correlations obtained have generally been good. However, some studies showed significant differences between analyzers[4,7~10]. Therefore, we compared measured FeNO levels between two analyzers, the stationary NOA 280i® analyzer and the hand-held NIOX MINO® analyzer. Furthermore, the formula of equation for conversion of values between the two analyzers was determined.

METHODS

Subjects

This study was conducted in 167 subjects, including outpatients who underwent FeNO measurement for diagnosis and management of asthma at the Pulmonary Medicine and Clinical Immunology Department of Dokkyo Medical University Hospital, outpatients presenting with cough to the same department who underwent FeNO measurement for differential diagnosis, and healthy volunteers. After informed consent was obtained from each subject, FeNO levels were measured sequentially using both a stationary analyzer and a hand-held analyzer.

Measurement of fractional exhaled nitric oxide

For measurement using the stationary analyzer (NOA280i®, Sievers Inc, USA), the subject was seated without a nose clip and placed the mouthpiece of the analyzer into the mouth. After breathing became stable, the patient exhaled for 5 seconds from maximum inspiration, while watching the display on the monitor and controlling the expiratory flow rate at 50 ml/s. Measurement was repeated three times, and the plateau FeNO concentration was determined on the monitor each time. Then the mean value of the three measurements was calculated as the measured FeNO level. For measurement using the hand-held analyzer (NIOX MINO®, Aerocrine Inc, Sweden), the subject was seated without a nose clip. The subject placed the mouthpiece of the analyzer into the mouth after exhaling completely, and then performed deep inspiration through the filter and subsequently exhaled the air slowly. The subject viewed a mirror reflecting the monitor of the analyzer and controlled the expiratory flow rate at 50±5 ml/s by keeping a picture of a cloud that moves depending on the expired air pressure within the blue area on the monitor (10−20 cmH_2O). As the subject exhaled for approximately 10 seconds, calculation of the FeNO level commenced automatically and was completed after approximately 100 seconds. The FeNO level calculated by the apparatus was used as the measured value. According to the directions of the manufacturer, measurement of FeNO with the NIOX MINO® was only performed once. The measurement with the NIOX MINO® analyzer was performed after measurement with the NOA280i® analyzer. This study was approved by the Ethics Commitiee of Dokkyo Medical University School of Medicine (hop-m22095) and was conducted in accordance with Declaration of Helsinki.

Statistical analysis

The correlation between FeNO values measured by the two analyzers was evaluated, and the correlation was considered to be significant if the p value was

less than 0.05. Based on the results of linear regression analysis, an equation for conversion of FeNO levels between the two analyzers was determined. Bland-Altman plot analysis was performed to evaluate the difference of measured FeNO values between the two analyzers. Results are presented as the geometric mean ± 95% confidence interval. Mean FeNO values were compared using a paired *t*-test. Statistical analysis was performed using JMP version 9.0.2 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

The mean age of the 167 subjects enrolled in this study (82 men and 85 women) was 48.6 years (range, 21－89 years). The baseline characteristics and smoking history of the subjects are shown in Table 1. There were 73 nonsmokers (43.7%), 59 ex-smokers (35.3%), and 19 current smokers (11.4%). The smoking history was unknown for 16 subjects (9.6%). The diseases of the patients are shown in Table 1. There were 108 patients with bronchial asthma (64.7%) and 12 patients with cough variant asthma (7.2%), and these two groups combined were approximately 70% of all subjects. In addition, there were 11 patients with COPD, 9 patients with allergic rhinitis, 7 patients with chronic cough, 5 patients with post-infectious cough, 2 patients with sarcoidosis, 9 healthy subjects, and 1 patient each with lung cancer, angina pectoris, gastroesophageal reflux disease (GERD), and idiopathic pulmonary fibrosis (IPF).

Table 1 Characteristics of the subjects

Total number of the subjects	167
Mean age (range 21-89)	48.6 (21-89)
Sex (male/female)	82/85
Smoking history	
never	73 (43.7%)
ex-	59 (35.3%)
current	19 (11.4%)
unknown	16 (9.6%)
Diseases	
Bronchial asthma	108 (64.7%)
(+allergic rhinitis)	58
(+sinusitis)	7
(+chronic eosinophilic pneumonia)	3
cough variant asthma	12 (7.2%)
COPD	11 (6.6%)
Allergic rhinitis	9 (5.4%)
Chronic cough	7 (4.2%)
Post infectious cough	5 (3.0%)
Sarcoidosis	2 (1.2%)
Others *	4 (2.4%)
Healthy	9 (5.4%)

* Lung cancer (n＝1), Angina pectoris (n＝1), gastroesophgaeal reflux disease (n＝1) idiopathic pulmonary fibrosis (n＝1). + : comorbidities related with allergic diseases.

The correlation between FeNO levels measured using the two analyzers is shown in Figure 1. The range of FeNO levels measured with the NOA280i® analyzer and the NIOX MINO® analyzer was 10.8－322.9 ppb and 10－182 ppb, respectively. The mean coefficient of variation (CV) of the three FeNO levels obtained at each time of measurement using the NOA280i® analyzer was 3.0% (0－11.7%), while the CV could not be calculated for data obtained with the NIOX MINO® analyzer because measurement was only performed once. Regression analysis was performed to evaluate the correlation between FeNO levels measured using the two analyzers, revealing a strong positive correlation between FeNO levels measured using the NOA280i® analyzer [FeNO (NOA 280i®)] and FeNO levels measured using the NIOX MINO® analyzer [FeNO (NIOX MINO®)] (r＝0.938, p＜0.0001). However, FeNO (NIOX MINO®) values were lower than FeNO (NOA280i®) values, being approximately 65－70% of the levels obtained with the NOA280i® analyzer. The following conversion equation was determined from the regression line : FeNO (NOA280i®) ×0.63＋3.79＝FeNO (NIOX MINO®). The correlations between the two analyzers were also determined for measurement at different FeNO levels across the range from low to high (Figure 2). This analysis revealed that the r value was 0.730 (p＜0.0001) when FeNO level (NOA280i®) was ≤50, while the r value was 0.641 (p＜0.0001) when FeNO level (NOA280i®) was 50 to ≤100, the r value was 0.607 (p＝0.0008) when FeNO level (NOA280i®) was 100 to ≤150, and the r value was 0.523 (p＝0.0180) when FeNO level (NOA280i®) was more than 150. When equivalence of the correlation coefficients was tested among the groups, no statistically significant differences were found and the difference was not significant even at the highest FeNO levels.

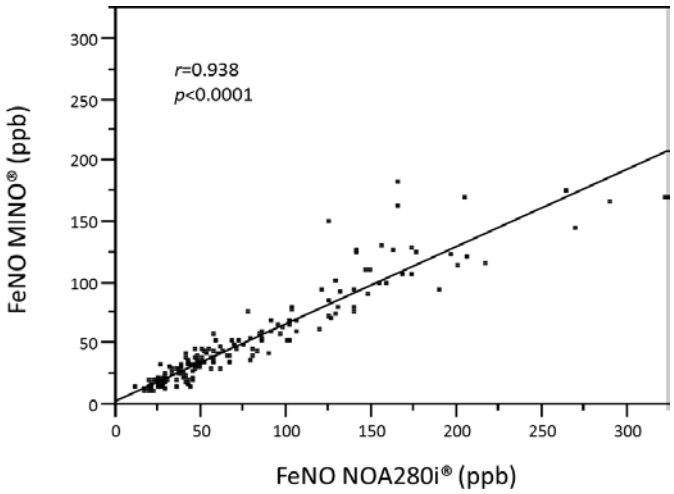

Figure 1

Correlation between FeNO levels measured using the NOA280i® and NIOX MINO® analyzers. Regression analysis showed that there was a strong and statistically significant correlation between data obtained using the two analyzers ($r=0.938$). However, increased divergence from the regression line was observed at high FeNO levels.

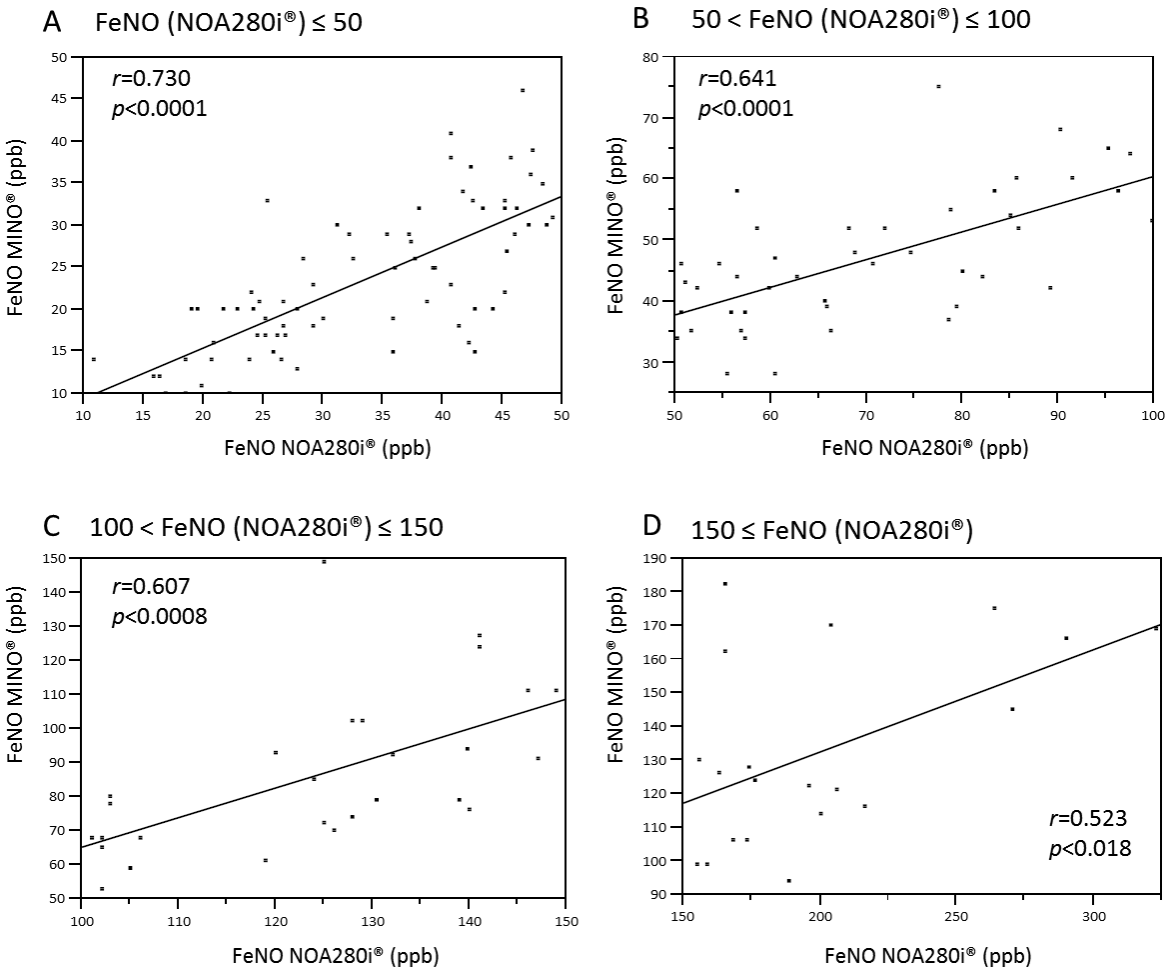

Figure 2

Correlation between measurements obtained using the NOA280i® and NIOX MINO® analyzers at different FeNO levels. The correlation became weaker as the FeNO level increased. The r value was 0.730 ($p<0.0001$) when FeNO level (NOA280i®) was ≤50 (A), while the r value was 0.641 ($p<0.0001$) when FeNO level (NOA280i®) was 50 to ≤100 (B), the r value was 0.607 ($p=0.0008$) when FeNO level (NOA280i®) was 100 to ≤150 (C), and the r value was 0.523 ($p=0.0180$) when FeNO level (NOA280i®) was more than 150 (D). A significant correlation was observed for each FeNO group, but the correlation became weaker as the FeNO level became higher.

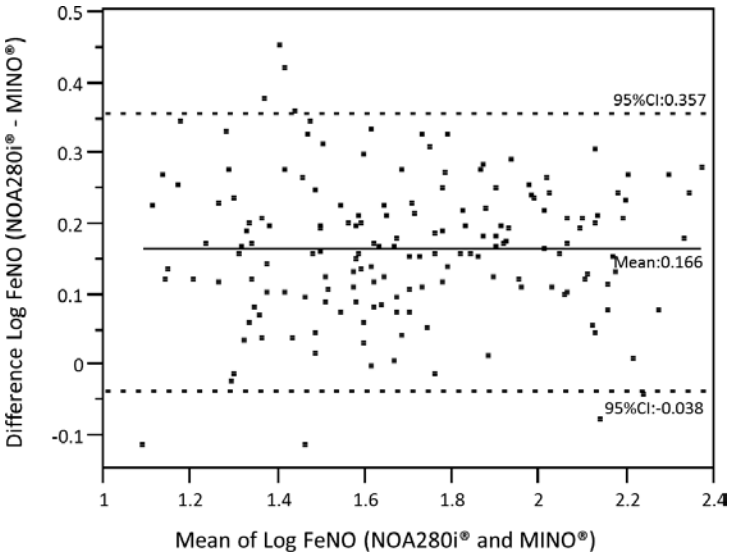

Figure 3

Bland-Altman plot of data obtained with the NOA280i® and NIOX MINO® analyzers. There was a positive difference of measured values between the two analyzers. The mean of the log-transformed FeNO values measured using the two analyzers is plotted on the X axis and the difference of the log-transformed FeNO values (NOA280i®-NIOX MINO®) is plotted on the Y axis. The geometric mean value of the difference between the two analyzers was calculated to be 0.167 (95% confidence interval = −0.038 to 0.357). Although positive difference of the measured values was observed between the two analyzers, the difference was not significant.

However, it was suggested that the correlation between data obtained with the two analyzers decreased as the FeNO level increased and the correlation was weakest at high FeNO levels exceeding 150.

Bland-Altman plot analysis was performed to evaluate the differences of measured values between the two analyzers, and the results are shown in Figure 3. The mean of the log-transformed FeNO values obtained using the two analyzers was plotted on the X axis and the difference of the log-transformed FeNO values (NOA280i® -NIOX MINO®) was plotted on the Y axis. The geometric mean of the difference in FeNO values between the two analyzers was 0.167 (95% confidence interval : −0.038 to 0.357), so there was a positive difference of the FeNO levels between the two analyzers. A comparison of the FeNO values obtained using the two analyzers (median and 95% confidence interval) is shown in Figure 4. The median FeNO level obtained with the NIOX

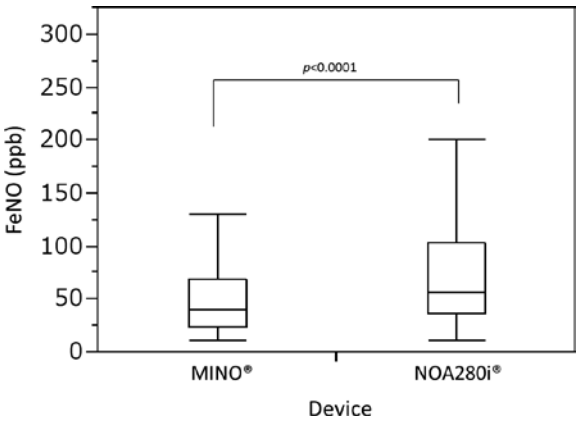

Figure 4

Comparison of FeNO levels obtained using the NOA 280i® and NIOX MINO® analyzers. FeNO levels measured using the NIOX MINO® analyzer were lower than those measured using the NOA280i® analyzer. The median FeNO level obtained with the NIOX MINO® analyzer was 39 (95% CI : 14−139) and the median FeNO level measured using the NOA280i® analyzer was 55.9 (95% CI : 19.6−198.4). There was a significant difference between the two analyzers (n =167, $p<0.0001$).

Table 2 Formulas to equation for conversion of values between devices.

Devices	Formulas to equation for conversion	References
NOA280i® vs NA623N®	FeNO NOA280i® = FeNO NA623N® × 0.994 − 0.431	(11)
NA623N® vs Nobreath®	FeNO NA623N® = FeNO NObreath® × 0.953 + 5.779	(12)
N-6008®vs NIOX MINO®	FeNO NIOX MINO® = FeNO N6008® × 1.5 + 10	(13)
Niox® vs NIOX MINO®	FeNO Niox® = FeNO NIOX MINO® × 0.808 − 1.656	(14)
NA623N® vs NIOX MINO®	FeNO NA623N® = FeNO MINO® × 1.278 + 3.065	(15)
NOA280i® vs NIOX MINO®	FeNO MINO® = FeNO NOA280i® × 0.63 + 3.79	this report

MINO® analyzer (39 ; 95% CI = 14 − 139) was significantly lower than that obtained by using the NOA280i® analyzer (55.9 ; 95% CI = 19.6 − 198.4, $p < 0.0001$).

DISCUSSION

This study demonstrated that FeNO levels measured using a portable analyzer (NIOX MINO®) were strongly correlated with those measured using a stationary analyzer (NOA280i®). However, the difference of measured values between the two analyzers increased as the FeNO level became higher and the correlation became weaker as the levels of FeNO exceeded 150 ppb. Bland-Altman plot analysis was also performed to evaluate the difference between the two analyzers, revealing that there was a positive difference of measured values between the two analyzers and that FeNO levels obtained using the NIOX MINO® analyzer were significantly lower (by approximately 65 − 70%) than those measured by the NOA280i® analyzer across the entire range of FeNO values.

Some earlier studies compared FeNO values between different analyzers and showed a strong correlation, with conversion equations being calculated based on the results of regression analysis. The conversion equations obtained in previous studies and the equations determined in the present study are shown in Table 2. It was reported that the FeNO levels obtained with two stationary analyzers (NA623NP® vs. NOA280i®)[11] were highly similar, as were the FeNO levels obtained with a stationary analyzer and a portable analyzer (NA623NP® vs. NObreath®)[12], while it was also reported that FeNO levels measured by using stationary analyzer N-6008® and Niox® were significantly lower than that obtained with the NIOX MINO® portable analyzer[13,14]. It was also reported that the FeNO level measured by using the NIOX MINO® analyzer was significantly lower than that obtained with the NA623NP® stationary analyzer[15]. Previous studies and the present study have both shown that the FeNO level measured using the NIOX MINO® portable analyzer is lower than that obtained with a stationary analyzer (NA623NP® or NOA280i®). There was a previous report that FeNO levels obtained using the NIOX MINO® portable analyzer were lower than those measured by using the NOA280i® stationary analyzer[16], which corresponds with our present results, but most of the subjects were healthy individuals in the previous study and the mean FeNO level was only around 20 ppb. These findings were supported by our results, since we found a strong correlation between the FeNO values obtained with the two analyzers the FeNO level was ≤50 and we also found that FeNO levels measured using the NIOX MINO® analyzer were lower.

Differences of the FeNO values obtained with the two analyzers in the present study may be explained by differences of the measurement principle and apparatus. A comparison of the specifications of the two analyzers is shown in Table 3 according to the manufacturer's instructions. The method of measurement (chemiluminescence method or ion electrode method), measurement conditions, and measurement range (1 − 500000 ppb with the NOA280i® analyzer vs. 5 − 300 ppb with the NIOX MINO® analyzer) are all different between them. While the previous report on patients with FeNO levels of 100 or less did not include an equation conversion of results between the two analyzers[16], we determined a conversion equation in our study as added on Table 2.

When the FeNO levels obtained by using the two

Table 3 Comparison of technical specifications between NOA280i® and NIOX MINO®

Device	NOA280i®	NIOX MINO®
Method	chemiluminescence	electrochemical
Optimal temperature	0～30℃	16～30℃
Optimal humidity	0～90%	20～60%
Measurable number of times/h	not written	10 measurements/h
Range	1～500,000 ppb	5～300 ppb
Weight	16 kg	0.8 kg
Shelf-life	—	Instrument : Minimum 3 years ant time of delivery or 1500 measurements Sensor : 15 months

analyzers were compared in this study, a considerable difference was observed between them. For example, the FeNO level of a patient was 322.9 ppb when measured using the NOA280i® analyzer and it was 169 ppb when measured using the NIOX MINO® analyzer. The difference of FeNO values between the two analyzers became larger at higher FeNO levels. In such a case, the correlation may not show a straight regression line like that in Figure 1, and it may instead be described by a downwardly convex quadratic curve. However, Bland-Altman plot analysis of the difference in measured values between the two analyzers revealed a constant positive difference between them at any FeNO level, and showed that the difference of values measured with the two analyzers did not increase as the mean FeNO level became higher. If difference of FeNO levels measured by the two analyzers had increased, the Bland-Altman plot should have shown a linear positive slope, but this was not demonstrated by the analysis in our study. Since there was a difference of the measurement range between the NOA280i® and NIOX MINO® analyzers, high FeNO levels over 300 measured using the NOA280i® analyzer are beyond the range of the NIOX MINO® analyzer, and this may have led to a difference of measured values between the two analyzers. In the present study, some of the subjects had FeNO levels greater than 100, but many of the other subjects had FeNO levels ≤100. Accordingly, we think that studies with a large number of patients with high FeNO levels (greater than 100) should be conducted in the future. We found that the difference of measured values between the two analyzers in patients with a high FeNO level did not influence the diagnosis of asthma. However, with regard to assessing the response to treatment, variation of FeNO levels measured by using the NIOX MINO® analyzer could make it difficult to evaluate the efficacy of treatment. Therefore, caution is needed when using the NIOX MINO® analyzer for assessing the therapeutic efficacy in patients with high FeNO levels.

Regarding the variation of FeNO levels when the measurement was repeated, according to the data in the manual for the NIOX MINO® analyzer, the variation is ± 5 ppb or up to ± 15% when measurement is performed once. However, it has been reported that the CV of FeNO levels measured using the NIOX MINO® analyzer was actually 18.3% [17]. In the present study, the CV of FeNO values measured using the NOA280i® analyzer was only 3.0% . Therefore, there may be a greater variation of the FeNO data obtained by using the NIOX MINO® analyzer. According to the data we obtained, the extent of variation compared with the stationary analyzer is ± 5 ppb when the FeNO level is ≤50 ppb, while the variation is ± 10 ppb when the FeNO level is 50 to ≤100 ppb, and the variation is ± 25 ppb when the FeNO level is more than 100 ppb. While such variation of the measured FeNO levels should be taken into consideration in the evaluation of measurement precision, it is unlikely to influence the difference of the measured values between the two analyzers seen in this study.

The order of measurement could also be a factor contributing to the difference of FeNO levels between the two analyzers in the present study since it has been shown that the FeNO level is lower when measured after respiratory function tests. In our study,

measurement with the NIOX MINO® analyzer was performed after measurement using the NOA280i® analyzer, and therefore the levels obtained with the NIOX MINO® might have been lower. However, measurement was performed three times with the NOA280i® analyzer and the mean value was used as the FeNO level in this study, but assessment of the individual measurements showed that the third measurement was not lower than the first measurement. Because measurement using the NIOX MINO® analyzer was performed immediately after measurement using the NOA280i® analyzer, we do not think that the order of the measurement caused the difference of FeNO levels between the two analyzers. To minimize potential bias related to the order of the measurement, measurement using NIOX MINO® should also have been performed three times and the two analyzers should have been used alternately. A stationary analyzer (NOA280i®), a gold standard analyzer, has been widely used for measurement of FeNO in clinical practice. In an official ATS clinical practice guideline recommendation, FeNO greater than 50 ppb is an indication of eosinophilic inflammation of patients with asthma[18]. The difference of FeNO levels between the two analyzers could become a problem in clinical practice when the FeNO measurement is near the cut-off value for asthma, since the difference could lead to false-negative or false-positive results, suggesting that caution is needed.

In conclusion, FeNO values measured with stationary and hand-held analyzers demonstrated a strong correlation, although a significant difference of measured values was observed between some analyzers. The compatibility of each pair of analyzers has been assessed and conversion equations have been determined. When comparison of the FeNO levels measured using different analyzers is performed in a multicenter trial or a meta-analysis, the conversion equations can be used. Our present findings suggested that the normal range of FeNO levels and the cut-off value for asthma should be established separately for each analyzer because of the difference in measured values between analyzers.

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Conflict of Interest

The authors declare that they have no conflct of interest.

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