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To cite this article: Junpei Saito, Masami Kikuchi, Atsuro Fukuhara, Suguru Sato, Mami Rikimaru, Yasuhito Suzuki, Manabu Uematsu, Naoko Fukuhara, Takaya Kawamata, Takeshi Umeda, Ryuichi Togawa, Yuki Sato, Tatsuhiko Koizumi, Kenichiro Hirai, Hiroyuki Minemura, Takefumi Nikaido, Kenya Kanazawa, Yoshinori Tanino, Yoko Shibata & Mitsuru Munakata (2019): Comparison of fractional exhaled nitric oxide levels measured by different analyzers produced by different manufacturers, Journal of Asthma, DOI: [10.1080/02770903.2019.1642351](https://doi.org/10.1080/02770903.2019.1642351)

To link to this article: <https://doi.org/10.1080/02770903.2019.1642351>



Accepted author version posted online: 09 Jul 2019.
Published online: 22 Jul 2019.



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Department of Pulmonary Medicine, School of Medicine, Fukushima Medical University, Fukushima, Japan

ABSTRACT

Objective: Fractional exhaled nitric oxide (FeNO) is widely used as a biomarker of allergic airway inflammation. At present, both stationary chemiluminescence and portable electrochemical analyzers produced by different manufacturers are available. However, it remains debatable whether those analyzers are comparable to each other. We compare FeNO levels obtained by different analyzers.

Methods: For the first study, 153 subjects were enrolled to compare differences in FeNO levels measured using three analyzers (NA623NP[®], NObreath[®], and NIOX MINO[®]) which were produced by different manufacturers. For the second study, 30 subjects were recruited to compare FeNO levels obtained by the two analyzers (NIOX MINO[®] and NIOX VERO[®]) produced by the same manufacturer. FeNO was measured twice using each analyzer in random order.

Results: FeNO levels obtained using the NIOX MINO[®] and NObreath[®] were more variable than those measured using the NA623NP[®]. There were strong positive correlations in FeNO levels measured by the NA623NP[®], NIOX MINO[®], and NObreath[®] ($p < 0.001$). The NA623NP[®] and NIOX MINO[®] provided the highest and lowest FeNO levels, respectively; whereas, those obtained by NObreath[®] were intermediate. No significant differences were observed in FeNO levels obtained using the NIOX MINO[®] and NIOX VERO[®].

Conclusions: FeNO levels measured by the NIOX MINO[®] and NIOX VERO[®], both of which were produced by the same manufacturer, have comparability. However, significant differences in FeNO levels exist when measured by analyzers manufactured by different manufacturers. This should be taken into account for FeNO measurement.

Abbreviations: ANOVA: analysis of variance; ATS: American Thoracic Society; CI: confidence interval; CV: coefficient of variation; FeNO: fractional exhaled nitric oxide; FVC: forced vital capacity; FEV₁: forced expiratory volume in one second; ppb: parts per billion

ARTICLE HISTORY

Received 14 November 2018

Revised 21 June 2019

Accepted 7 July 2019

KEYWORDS

Exhaled nitric oxide; chemiluminescence analyzer; electrochemical analyzer; NIOX MINO[®]; NObreath[®]; NIOX VERO[®]; device difference

Introduction

Asthma is a chronic airway inflammatory disorder characterized by reversible airflow obstruction and non-specific airway hyperresponsiveness (1). Consequently, monitoring airway inflammation, which is the fundamental pathophysiology of asthma, is important not only for the diagnosis, but also for the control of asthma. Fractional exhaled nitric oxide (FeNO) measurement is now becoming widely incorporated into routine clinical practice as a convenient, sensitive, and noninvasive method of monitoring allergic airway

inflammation. Many previous reports have shown that FeNO levels are increased in patients with asthma and cough variant asthma (2–5), and decreased in response to corticosteroid therapy (5–7). Therefore, FeNO can be a useful noninvasive marker for the diagnosis and management of asthma.

Two types of FeNO analyzers, known as stationary chemiluminescence and portable electrochemical analyzers are currently used in both research and clinical settings. Of those, stationary chemiluminescence analyzers are well-validated for online FeNO measurement (8).

The advantages of the stationary devices are that they are highly responsive, sensitive, and specific for nitric oxide measurements. However, they are expensive, not portable, and require frequent calibration. Therefore, to date, they have been mostly used in research settings and may not be applicable to routine clinical practice. To overcome these disadvantages, portable electrochemical analyzers have been developed and validated for FeNO measurements (9–19). The major advantages of these devices are that they are small, easy to use, and relatively inexpensive to acquire, and their running cost is low (20,21).

At present, only FeNO measurements yielded by portable analyzers known as NIOX MINO[®] (Circassia, Solna, Sweden), NIOX VERO[®] (Circassia, Solna, Sweden) and NObreath[®] (Bedfont Scientific Ltd., Rochester, Kent, UK), are covered by National Health Insurance in Japan. The NIOX VERO[®] is newer than the NIOX MINO[®] and they are produced by the same manufacturer. The former has recently replaced the latter in Japan, as well as in Europe and the USA. However, little investigation has been made into whether these three devices can be comparable to each other. Additionally, it remains debatable whether FeNO levels obtained by these portable analyzers are equivalent to those measured by stationary chemiluminescence analyzers such as the NOA280i[®] (Sievers Co., Boulder, CO, USA) and NA623NP[®] (CHEST M.I., Inc., Tokyo, Japan) (22).

The aims of the study were as follows. Firstly, to evaluate whether FeNO levels measured by the three analyzers (NIOX MINO[®] vs NObreath[®] vs NA623NP[®]) produced by the different manufacturers are reproducible and comparable to each other. The second aim was to determine whether FeNO level measured by NIOX VERO[®] is interchangeable with those obtained by the NIOX MINO[®], both of which are developed by the same manufacturer.

Material and methods

Study design

This is a single-center, open-label, observational study. For Study 1, FeNO levels were measured using three different manufactured devices (NIOX MINO[®], NObreath[®], and NA623NP[®]) in random order, and measured FeNO levels were compared. For Study 2, FeNO measurements were performed randomly using the NIOX MINO[®] and NIOX VERO[®] to demonstrate whether they are clinically equivalent, because the former has been replaced with the latter.

Subjects

For the first study, 103 asthmatic subjects and 29 non-asthmatic subjects with respiratory disorders were recruited from the Department of Pulmonary Medicine at Fukushima Medical University Hospital from August 2015 to May 2016. Twenty-one healthy volunteers with normal spirometry and no respiratory disorders were also recruited from the staff at Fukushima Medical University in June 2016. All cases of asthma were diagnosed in accordance with the Japanese Guideline for Adult Asthma 2014 (23). Twenty-six of the 103 asthmatic subjects were newly diagnosed as having asthma at enrollment, and the remaining 77 subjects were treated with inhaled corticosteroid either alone or with other anti-asthmatic drugs. Non-asthmatic disorders comprised five cases of atopic cough, five cases of chronic obstructive pulmonary disease, four cases of post nasal drip, four cases of interstitial lung disease, three cases of sinobronchial syndrome, three cases of post infectious cough, two cases of lung cancer, one case of gastroesophageal reflux, one case of acute bronchitis, and one case of psychogenic cough.

For the second study, 26 asthmatic subjects and four non-asthmatic subjects with respiratory disorders (two cases of post infectious cough and two case of sinobronchial syndrome) were enrolled from the same clinic for the comparison of FeNO levels measured using the NIOX MINO[®] and NIOX VERO[®] from August to November in 2017. All subjects were stable under appropriate treatment.

This study was approved by the Local Ethics Committee of Fukushima Medical University (NO. 321, November 2014), and only subjects who had given written informed consent were enrolled.

FeNO measurement

Technical information of the FeNO analyzers used in this study are shown in Table 1. FeNO measurements were conducted in line with the recommendations of the American Thoracic Society (ATS)/European Respiratory Society (24). Measurements using the stationary chemiluminescence analyzer (NA623NP[®]) were performed under resting ventilation, with the subjects in a sitting position, not wearing a nose clip. The subjects exhaled at a constant flow rate (50 ml/s), from total lung capacity to residual volume, for 10 s without holding their breath, while they maintained a constant mouth pressure (16 cm H₂O). The participants ensured that these conditions were met by monitoring a visual display to eliminate contamination from nasal NO. The NA623NP[®] is calibrated once a

Table 1. Device comparison.

	NA623NP [®]	NO breath [®]	NIOX MINO [®]	NIOX VERO [®]
Measurement range (ppb)	1–5000	5–300	5–300	5–300
Principle	Chemiluminescence	Electrochemical	Electrochemical	Electrochemical
Accuracy	<50 ppb: ± 2.5 ppb ≥50 ppb: ± 5 %	<50 ppb: ± 5 ppb ≥50 ppb: ± 10 %	<50 ppb: ± 5 ppb ≥50 ppb: ± 10 %	<50 ppb: ± 5 ppb ≥50 ppb: ± 10 %
Visual feedback	Computer screen	Display	Display via mirror	Display
Calibration on site	Yes	No	No	No
Interval for calibration	Once a week	Once a year at a distributor	Disposable device (3 years)	Disposable device (5 years)
Dimension (cm)	20 × 40 × 40	15 × 9 × 5	24 × 13 × 10	19 × 15 × 4
Weight (kg)	20	0.4	0.8	1.0

week using standard calibration gas containing 500 parts per billion (ppb) of NO, in accordance with the manufacturer's instruction.

For measurements using the portable electrochemical analyzers (NIOX MINO[®] and NIOX VERO[®]), subjects were asked to empty their lungs, then to inhale nitric oxide-free air deeply, to total lung capacity, through a mouthpiece. Finally, while looking at a cloud floating in the gray zone or moving upwards on a monitor and listening to bleeps, the subjects were instructed to exhale their breath into the analyzer at a constant exhalation flow rate of 50 ml/s. Neither the NIOX MINO[®] nor the NIOX VERO[®] require any calibration, according to the manufacturer's instructions.

For measurements of another portable electrochemical analyzer (NObreath[®]), the subjects first inhaled ambient air to total lung capacity and then, while keeping a small ball between the middle of the white band seen in the flow indicator, exhaled into the device through a mouthpiece at a standardized exhalation flow rate of 50 ml/s for about 10 s. NObreath[®] is calibrated once a year as part of a regular maintenance service provided by the manufacturer.

Quality control assessments for all devices are undertaken every day by confirming FeNO levels obtained from qualified staff members in our department.

FeNO was measured twice using each analyzer in random order. Spirometry was performed after all FeNO measurements. Each FeNO level was assessed for reproducibility. The tolerance limits were defined as differences of <10 ppb for absolute levels of ≤50 ppb, and of <20% for those >50 ppb, in line with the ATS guidelines (5). If the two measurements did not fulfill the above-mentioned criteria, the data were excluded from the analyses. The mean FeNO levels of two successful recordings were retained for comparison among the distinct analyzers.

Statistical analysis

Statistical analyses were performed using SPSS for Windows (version 21.0.1: SPSS, Chicago, IL, USA)

and Graph Pad Prism (version 5, Graph Pad Prism, San Diego, CA, USA). Data were expressed as mean or geometrical mean with 95% confidence intervals (CIs) when appropriate. Bland and Altman plots (25) were constructed to assess the within-subject reproducibility of FeNO levels using the first and second readings from each device and to compare FeNO levels measured by different devices. Data which did not meet the tolerance limits mentioned above were excluded from further analyses of comparisons among the different FeNO devices. As FeNO levels exhibited a non-normal distribution (Kolmogorov-Smirnov test), Spearman's rank correlation coefficients were calculated for comparisons among the devices. Differences in FeNO levels measured by the three devices (NIOX MINO[®], NObreath[®], and NA623NP[®]) were assessed in the whole range as well as the three ranges (<25, 25–50, 50 ppb<) based on the ATS-recommended FeNO cutoff levels (5) using the Friedman test with Dunns correction, and analysis of the differences in FeNO levels measured by the NIOX MINO[®] and NIOX VERO[®] was performed using the Mann Whitney U test. A two-tailed *p* values of 0.05 or less was considered statistically significant.

Results

Baseline characteristics of study 1 and 2

The subjects' characteristics of Study 1 and 2 are shown in Table 2 and Table 3, respectively. In Study 1, both the asthmatic and non-asthmatic subjects were significantly older than the healthy volunteers (*p* < 0.01). There were no significant differences in height or weight among the three groups. The forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) levels in the non-asthmatic subjects were significantly lower compared to those in the healthy subjects (*p* < 0.01). FeNO levels obtained by all three devices in the asthmatic subjects were significantly higher than those in the non-asthmatic and healthy subjects (*p* < 0.01). In Study 2, FeNO levels

Table 2. Subjects' baseline characteristics in study 1.

	Total (n = 153)	Asthmatic subjects (n = 103)	Non-asthmatic subjects (n = 29)	Healthy subjects (n = 21)
Sex (M/F)	76/77	55/48	11/18	10/11
Age (year)	53.9 (51.4–56.4)	56.4 (53.6–59.2) ^a	57.1 (51.4–62.8) ^a	37.2 (31.3–43.1)
Height (cm)	161.4 (160.0–162.7)	162.0 (160.3–163.7)	158.4 (155.5–161.3)	162.5 (158.7–166.4)
Weight (kg)	61.8 (59.8–63.7)	63.1 (60.6–65.6)	58.8 (53.9–63.6)	59.4 (55.3–63.4)
FVC (L)	3.26 (3.13–3.40)	3.31 (3.14–3.48)	2.94 (2.69–3.18) ^a	3.61 (3.23–4.00)
FVC(%pred) (%)	102.3 (99.4–105.1)	102.3 (98.9–105.7)	98.9 (91.9–105.8)	109.6 (100.4–118.8)
FEV ₁ (L)	2.39 (2.27–2.52)	2.41 (2.26–2.55)	2.15 (1.89–2.41) ^a	2.86 (2.48–3.24)
FEV ₁ (%pred) (%)	90.5 (87.4–93.6)	90.5 (87.1–93.9)	88.6 (78.8–98.3)	95.0 (87.0–103)
FEV ₁ /FVC (%)	73.4 (71.3–75.5)	72.8 (70.5–75.1)	73.0 (66.8–79.2)	78.8(74.1–83.5)
FeNO (ppb)				
NA623NP [®]	52.6 (44.8–60.4)	65.6 (55.0–76.2) ^{a, b}	25.6 (20.6–30.6)	25.8 (20.4–31.2)
NIOX MINO [®]	42.7 (35.9–49.4)	53.8 (44.5–63.0) ^{a, b}	20.2 (16.3–24.1)	19.2 (15.0–23.3)
NObreath [®]	47.0 (39.3–54.8)	59.1 (48.5–69.8) ^{a, b}	24.2 (18.7–29.7)	19.1 (14.3–24.0)

^a $p < 0.01$ (vs healthy control).^b $p < 0.01$ (vs non-asthmatic subjects).

Data are shown as mean and 95% confidence intervals.

Table 3. Subjects' baseline characteristics in study 2.

	Total (n = 30)	Asthmatic subjects (n = 26)	Non-asthmatic subjects (n = 4)
Sex (M/F)	15/15	12/14	3/1
Age (year)	60.5 (54.7–66.3)	59.6 (53.5–65.7)	66.5 (34.5–98.5)
Height (cm)	159.0 (155.9–162.2)	158.7 (155.3–162.1)	161.2 (146.1–176.4)
Weight (kg)	60.8 (56.4–65.1)	60.8 (56.0–65.7)	60.4 (46.6–75.2)
FVC (L)	3.13 (2.90–3.35)	3.15 (2.90–3.40)	3.00 (2.31–3.68)
FVC(%pred) (%)	103.8 (98.1–109.5)	105.1 (99.1–111.0)	95.6 (64.0–127.0)
FEV ₁ (L)	2.23 (2.06–2.41)	2.26 (2.07–2.45)	2.08 (1.18–2.97)
FEV ₁ (%pred) (%)	90.1 (84.4–95.9)	91.7 (86.0–97.3)	85.0 (64.7–99.8)
FEV ₁ /FVC (%)	72.0 (68.1–75.8)	72.5 (68.3–76.6)	68.5 (60.6–86.3)
FeNO (ppb)			
NIOX MINO [®]	50.0 (28.8–71.1)	54.1 (29.9–78.2) ^a	23.4 (2.78–44.0)
NIOX VERO [®]	52.5 (28.7–76.3)	56.3 (29.0–83.6) ^a	27.8 (0.00–57.5)

^a $p < 0.01$ (vs non-asthmatic subjects).

Data are shown as mean and 95% confidence intervals.

obtained by the NIOX MINO[®] and NIOX VERO[®] in the asthmatic subjects were significantly higher compared to those in the non-asthmatic subjects ($p < 0.01$).

Study 1

Variation of FeNO levels in each device

The Bland and Altman plots in Figure 1 show the differences between the 1st and 2nd measurements of each device. The average differences (95% limits of agreement) between the two repeated measurements by the NA623NP[®], NIOX MINO[®], and NObreath[®] were 0.04 ppb (−5.12 ppb, 5.19 ppb), −1.38 ppb (−13.6 ppb, 10.8 ppb), and −1.75 ppb (−16.9 ppb, 13.4 ppb), respectively. The NA623NP[®] (chemiluminescence analyzer) yielded better reproducibility compared to the NIOX MINO[®] and NObreath[®] (portable electrochemical analyzer). According to the ATS guidelines, FeNO levels in all subjects for NA623NP[®] measurement were within the tolerance limits. However, FeNO levels in five subjects (3.3%) for NIOX MINO[®] measurement and 11 subjects (7.2%) for NObreath[®] measurement were not within the tolerance limits, and were therefore

excluded from further analyses. The remaining 137 subjects got up to the next analyses.

Correlations among the three analyzers

There were significant correlations in FeNO levels measured by the three devices (NA623NP[®] vs NIOX MINO[®], $r = 0.956$, $p < 0.001$; NA623NP[®] vs NObreath[®], $r = 0.928$, $p < 0.001$; NIOX MINO[®] vs NObreath[®], $r = 0.948$, $p < 0.001$) (Figure 2).

Differences among the three analyzers

The geometric mean FeNO level obtained by the NA623NP[®] (37.0 ppb [44.8 ppb, 60.4 ppb]) was the highest, and that obtained using the NIOX MINO[®] (29.1 ppb [25.5 ppb, 33.2 ppb]) was the lowest, and that obtained using the NObreath[®] (30.9 ppb [26.7 ppb, 35.7 ppb]) was intermediate, with significant differences among the three analyzers ($p < 0.001$) (Figure 3). Furthermore, the performance of the devices was evaluated according to the three ranges of FeNO levels (<25, 25–50, 50 ppb<) based on the ATS-recommended FeNO cutoff levels. The geometric mean FeNO level obtained by the NA623NP[®] was

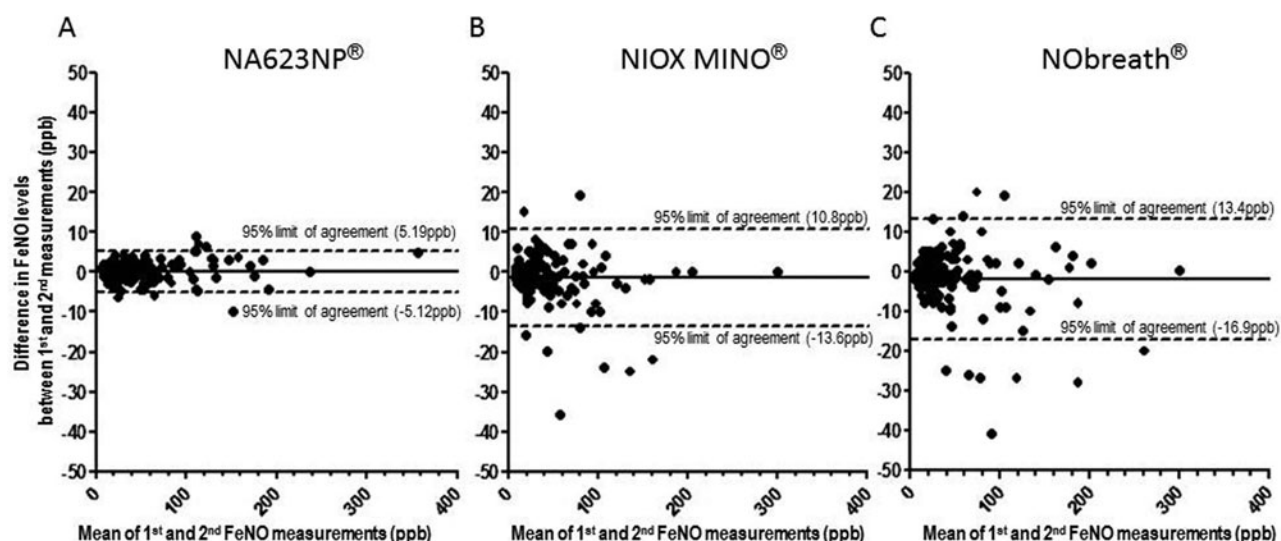


Figure 1. Bland and Altman plots of repeated measurements of fractional exhaled nitric oxide (FeNO) using the NA623NP[®] (A), NIOX MINO[®] (B), and NObreath[®] (C). The solid and dashed lines represent mean and 95% limits of agreement, respectively.

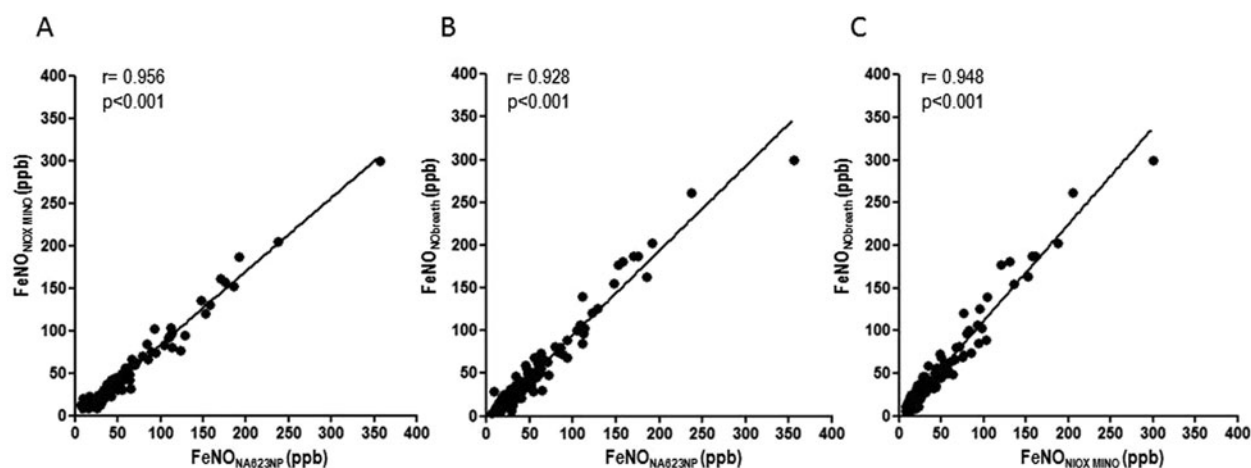


Figure 2. Correlation of fractional exhaled nitric oxide (FeNO) measured using (A) the NA623NP[®] and NIOX MINO[®], (B) the NA623NP[®] and NObreath[®], and (C) the NIOX MINO[®] and NObreath[®].

significantly higher compared to those obtained by the NIOX MINO[®] or NObreath[®] over all three ranges of FeNO levels ($p < 0.01$). Significant differences in geometric mean FeNO levels obtained by the NIOX MINO[®] and NObreath[®] could only be seen if the levels were 25 ppb or more ($p < 0.05$) (Figure 4).

Variations of FeNO levels among the three devices

When intra-subject mean FeNO levels were ≤ 50 ppb between any two of the three devices, 95% limits of agreements were within the range of ± 20 ppb, which is considered an acceptable agreement (Figures 5A–5C). On the other hand, when they were > 50 ppb, 95% limits of agreements were over the range of ± 20 ppb (Figures 5D–5F), indicating the existence of

intra-subject differences. Furthermore, even when subjects whose intra-subject difference in FeNO levels was ± 20 ppb or more were excluded, there were still significant differences in FeNO level measurements among the three devices (data not shown).

Study 2

Correlation and difference between NIOX MINO[®] and NIOX VERO[®]

FeNO levels in all subjects for NIOX MINO[®] and NIOX VERO[®] measurements were within the tolerance limits. Thus, all data were included in the analysis. There was a strong correlation in FeNO levels obtained by the NIOX MINO[®] and NIOX VERO[®] ($r = 0.972$, $p < 0.001$) (Figure 6A). Additionally, the

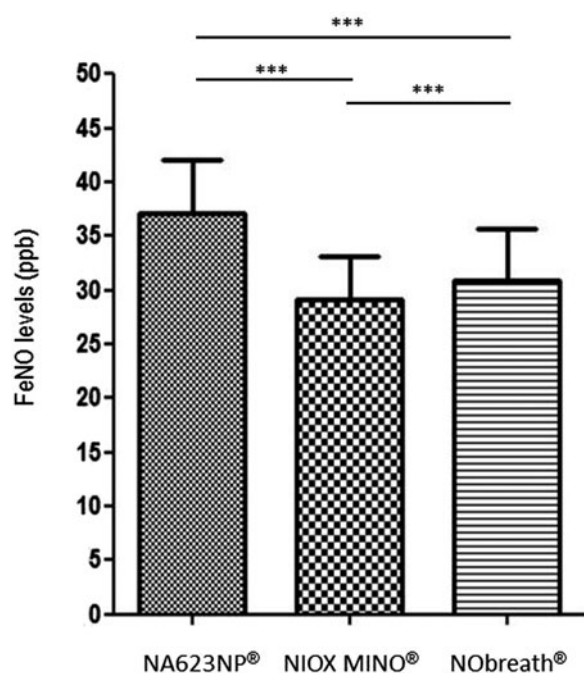


Figure 3. Comparison of fractional exhaled nitric oxide (FeNO) measured by the three analyzers (NA623NP®, NIOX MINO®, and NObreath®) produced by the different manufacturers. Boxes represent the geometric mean and the whiskers indicate 95% confidence interval. *** $p < 0.001$.

geometric mean FeNO levels obtained using the NIOX MINO® and NIOX VERO® were 32.7 ppb (95% CI: 23.0 ppb, 46.4 ppb) and 33.4 ppb (95% CI: 23.2 ppb, 48.0 ppb), respectively, showing no significant difference (Figure 6B). When intra-subject mean FeNO levels were ≤ 50 ppb between the NIOX MINO® and NIOX VERO®, the 95% limits of agreement were in the range of -8.15 – 7.20 ppb (Figure 6C), which was smaller than those among the devices produced by different manufacturers in Study 1. However, when intra-subject mean FeNO levels were > 50 ppb between the NIOX MINO® and NIOX VERO®, the 95% limits of agreement were wider (-52.2 – 39.2 ppb), which were similar to those in Study 1 (Figure 6D). Furthermore, even when the subjects whose intra-subject difference in FeNO levels was ± 20 ppb or more were excluded, there were still no significant differences in FeNO levels between these two devices (data not shown). This suggests that FeNO levels obtained by devices of the same manufacturer are similar to one another.

Discussion

The present study evaluated not only the within-subject repeatability of FeNO levels but also the

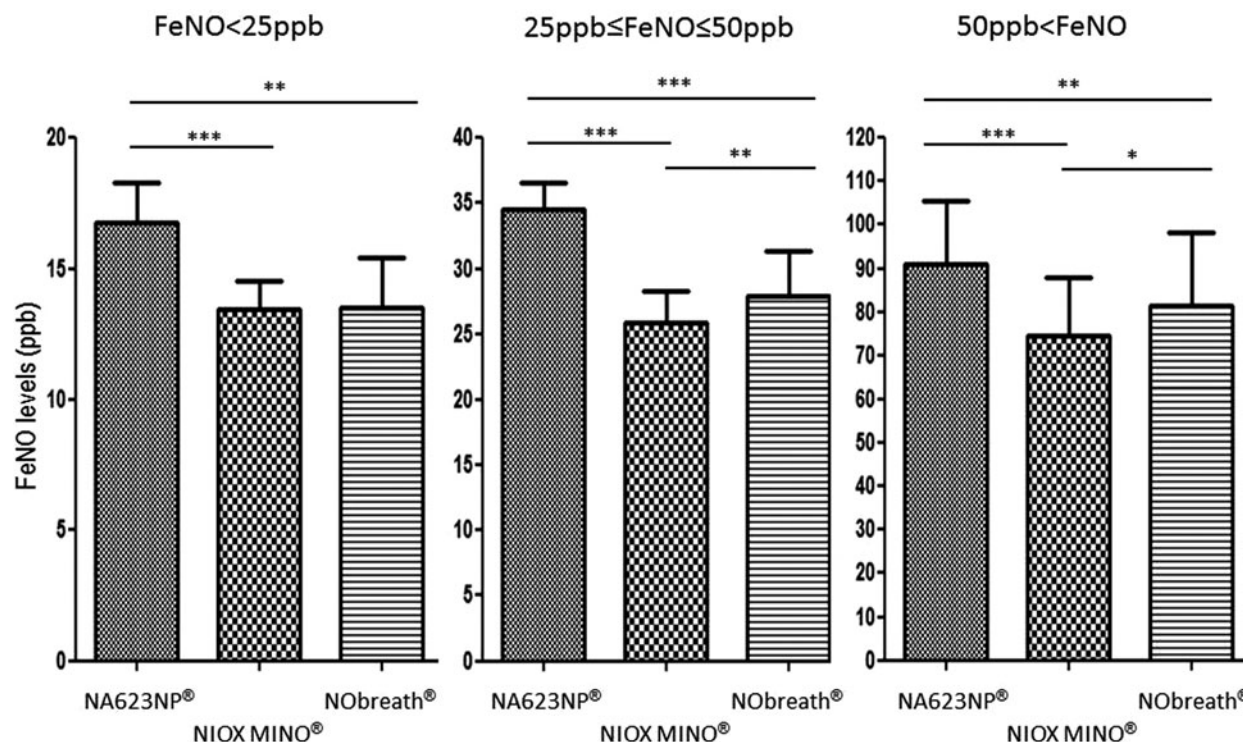


Figure 4. Performance of the analyzers (NA623NP®, NIOX MINO®, and NObreath®) produced by the different manufacturers were evaluated according to the three ranges of FeNO levels (< 25 , 25 – 50 , 50 ppb) based on the ATS-recommended cutoff levels. Boxes represent the geometric mean and the whiskers indicate 95% confidence interval of the mean. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

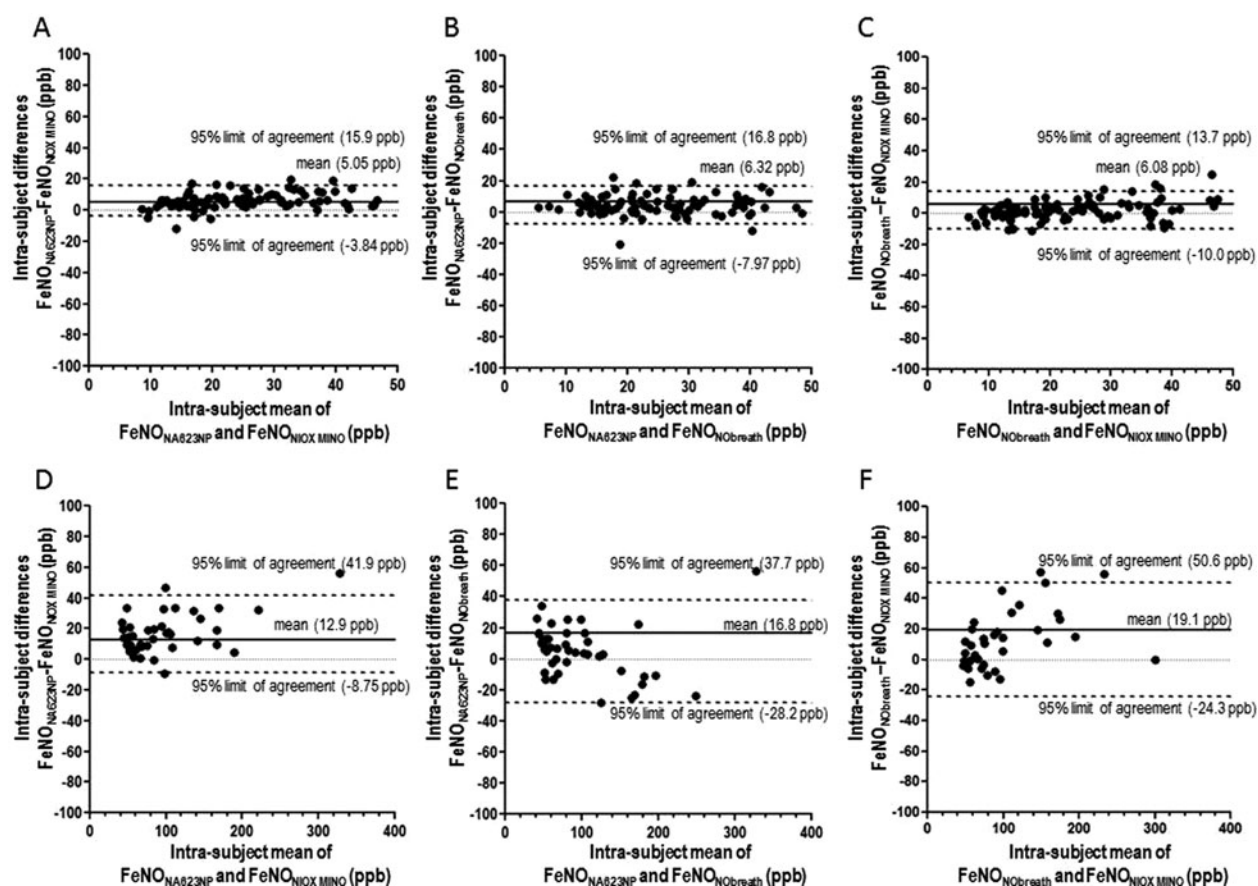


Figure 5. Bland and Altman plots of the intra-subject differences in fractional exhaled nitric oxide (FeNO) levels between the NA623NP[®] and NIOX MINO[®] (A), between the NA623NP[®] and NObreath[®] (B), and between the NIOX MINO[®] and NObreath[®] (C) when intra-subject mean FeNO levels were ≤ 50 ppb between any two of the three devices. Bland and Altman plots of the intra-subject differences in fractional exhaled nitric oxide (FeNO) levels between the NA623NP[®] and NIOX MINO[®] (D), between the NA623NP[®] and NObreath[®] (E), and between the NIOX MINO[®] and NObreath[®] (F) when intra-subject mean FeNO levels were > 50 ppb between any two of the three devices. The solid and dashed lines represent mean and 95% limits of agreement, respectively.

differences in FeNO levels measured by different analyzers. The results showed that FeNO levels obtained by the portable electrochemical analyzers were more variable compared to those measured by a stationary chemiluminescence analyzer. There were significant differences in FeNO levels measured by the devices of the different manufacturers (NA623NP[®], NObreath[®], and NIOX MINO[®]); whereas, FeNO levels measured by those of the same manufacturer (NIOX MINO[®] and NIOX VERO[®]) were similar.

Reproducibility of FeNO levels between the different analyzers

In the current study, Bland and Altman plots demonstrated good agreement between the repeated FeNO measurements using the NA623NP[®], NIOX MINO[®], and NObreath[®] (Figure 1). However, the 95% limits of agreement for both the NIOX MINO[®] and NObreath[®] were approximately twice as wide as that for the

NA623NP[®]. These variabilities did not change with higher FeNO levels. These results were similar to those of previous studies, which showed that the coefficient of variation (CV) percentages for the NIOX MINO[®], NObreath[®], and stationary chemiluminescence analyzers were in the range of 6.1 to 18.3% (11,12,14,26), 11.8% (15), and less than 3% (27,28), respectively. Therefore, stationary chemiluminescence analyzers are likely to be more accurate than portable electrochemical analyzers. As suggested in the ATS guidelines, in order to obtain accurate readings, two or more serial measurements are recommended for each patient, especially when using portable FeNO analyzers (24).

Comparison of FeNO levels between the electrochemical and chemiluminescence analyzers

In the current study, the FeNO levels measured with the NA623NP[®], NIOX MINO[®], and NObreath[®] showed strong correlations with one another. The FeNO levels

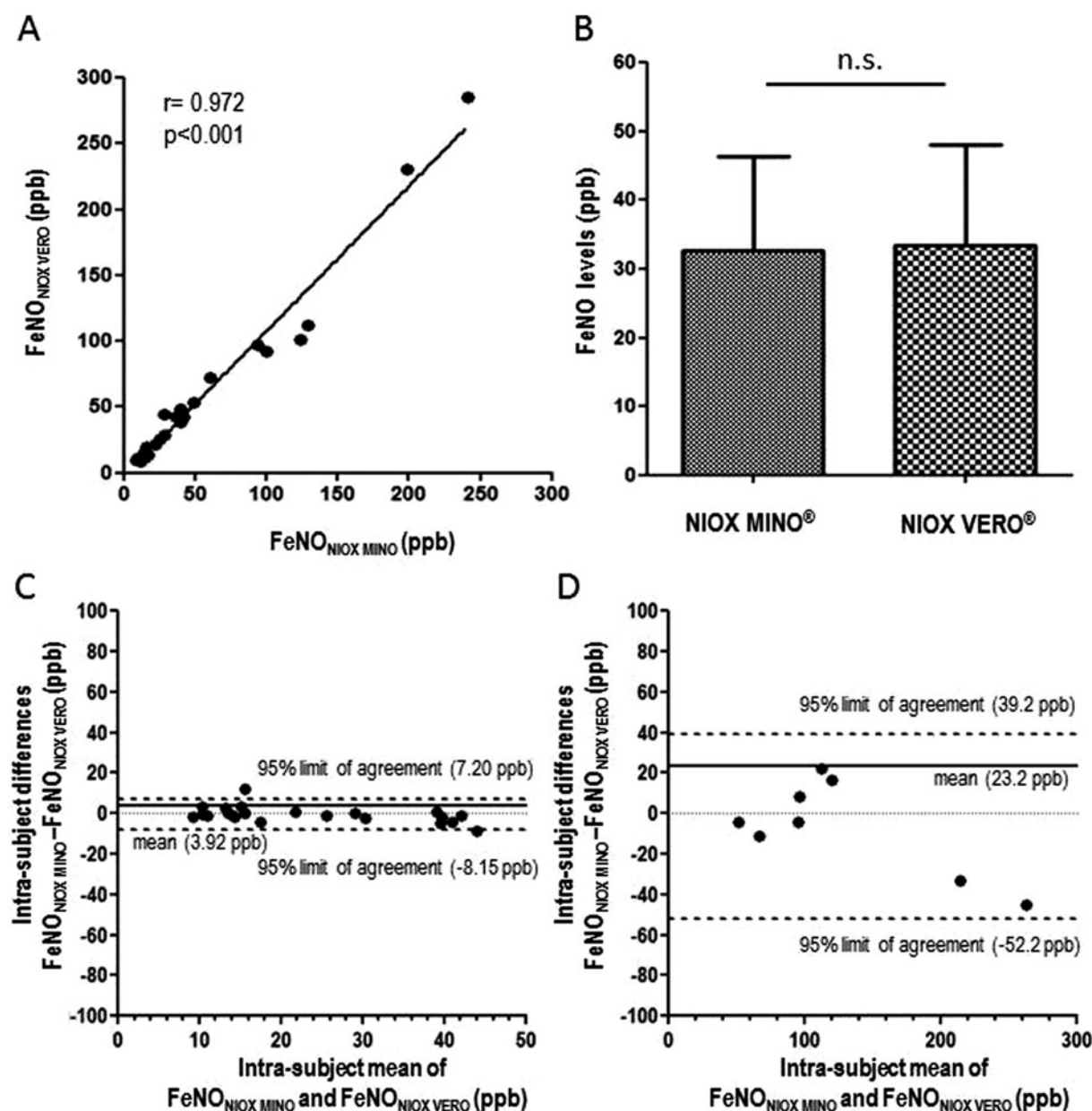


Figure 6. Correlation of fractional exhaled nitric oxide (FeNO) between the NIOX MINO[®] and NIOX VERO[®] (A). Comparison of FeNO measured by these two analyzers (B). Bland and Altman plot of differences in FeNO levels between the NIOX MINO[®] and NIOX VERO[®] in the subjects whose mean FeNO levels were ≤ 50 ppb (C). Bland and Altman plot of differences in FeNO levels between the NIOX MINO[®] and NIOX VERO[®] in the subjects whose mean FeNO levels were > 50 ppb (D). Boxes represent the geometric mean and the whiskers indicate 95% confidence interval of the mean. The solid and dashed lines represent mean and 95% limits of agreement, respectively.

obtained using the NA632NP[®] were the highest, those obtained by the NIOX MINO[®] were the lowest, and those obtained by the NObreath[®] were intermediate, with significant differences among all three analyzers, especially when regarding FeNO levels of 25 ppb or more. These findings are consistent with those from most previous studies including our previous study, which compared the NIOX MINO[®] with other stationary chemiluminescence analyzers (the NA632NP[®], NIOX[®], Ecomedics[®], and Loagan[®]) (9–14,17,19,26). All

these studies indicated strong positive correlations between NIOX MINO[®] and the stationary analyzers. At the same time, however, there has been controversy regarding the comparison of FeNO levels measured by different analyzers; some studies have reported that FeNO levels obtained by stationary analyzers were higher than those measured with the NIOX MINO[®] (11,17,19), while others revealed that FeNO levels were approximately the same (9,12,13,26), and yet others stated that FeNO levels from stationary analyzers were slightly lower

(10,14). Similarly, in another comparison, FeNO levels measured using the NObreath[®] were lower than those obtained by the stationary chemiluminescence analyzers (15,18). The cause of the differences in FeNO levels between electrochemical analyzers and chemiluminescence analyzers remains unclear. However, feasible causes could at least partly be explained by; (1) different principles of measurement; (2) different concentrations of calibration gas recommended by each manufacturer; (3) differences in measurement ranges; (4) variations in oropharyngeal pressure; and (5) variations in exhalation time and FeNO plateau levels recorded by each analyzer. Further investigations are needed to clarify the differences between these analyzers.

Comparison of FeNO levels measured by the portable electrochemical analyzers (NIOX MINO[®] vs NObreath[®]) produced by different manufacturers

A few studies have reported differences in FeNO levels. Direct comparisons of FeNO levels measured by the NIOX MINO[®] and NObreath[®] have shown conflicting results (15,16). Antus et al. described that FeNO levels measured using the NObreath[®] were higher than those measured using the NIOX MINO[®] (15), while Kapande et al. demonstrated that the NIOX MINO[®] produced higher results than the NObreath[®] (16). Our results indicating higher FeNO levels obtained by the NObreath[®] than the NIOX MINO[®] are in line with those reported by Antus et al. (15). The reason for this conflicting result remains unclear; however, there are several possible explanations. Firstly, the order in which FeNO levels were tested was different. Antus et al. measured FeNO levels using different devices in random order, while Kapande et al. measured them in the order of NIOX MINO[®] then NObreath[®]. Secondly, the enrolled subjects were different; the former study included only healthy adult subjects whereas the latter one included asthmatic children and healthy children. Lastly, the devices produced by different manufacturers may have influenced the results. In brief, no recording is made if a poor technique is employed while using the NIOX MINO[®], whereas the NObreath[®] will report FeNO levels regardless of technique.

Comparison of FeNO levels measured with analyzers by the same manufacturer (NIOX MINO[®] vs NIOX VERO[®])

There were no significant differences in FeNO levels between the NIOX MINO[®] and NIOX VERO[®], both

of which were produced by the same manufacturer, even when subjects with a wide range of intra-subject differences in FeNO levels were excluded. To date, there has only been one study comparing FeNO levels between the NIOX MINO[®] and NIOX VERO[®], producing results of which were consistent with our current study (29). Therefore, the data obtained using the NIOX MINO[®] in the previous studies have comparability with those obtained by the NIOX VERO[®].

Limitations

There were some limitations to the study. Firstly, the sample size was small in Study 2. Secondly, this was a single center study. A further multicentre study with a large population can reveal intra-operator and biological variability. Thirdly, intra-subject differences in FeNO levels are more likely to be detected, especially when FeNO levels were >50 ppb. The reason for this remains unclear. However, this can be partly explained by the differences in sensor sensitivity among the devices for detecting high levels of FeNO. This should also be evaluated by further studies that enroll subjects with FeNO levels higher than 50 ppb. Finally, the conversion equations for FeNO levels obtained using the different devices were not provided. We do not believe that the conversion equation for each device is useful because they could vary depending on study population and variability in FeNO measurements, as previously suggested (16).

Conclusion

In conclusion, FeNO levels produced by the same manufacturer are similar. However, significant differences in FeNO levels (especially when FeNO levels are ≥ 25 ppb) exist when measured using analyzers developed by different manufacturers or analyzers whose principles are different. This should be taken into account for FeNO measurement. Consistent use of the same well-validated analyzer is recommended for regular monitoring of FeNO levels in routine clinical practice for asthma management.

Acknowledgments

We would like to thank the Scientific English Editing Section in our university for their linguistic assistance in proofreading the manuscript. We would also like to thank Miss Yasuko Sato for the technical support.

Declaration of interest

All authors declared no conflict of interest.

Funding

No financial support was provided for the conduct of the research and/or preparation of this article.

Notes on contributors

JS conceived and designed the study; analyzed and interpreted the data; drafted and edited the manuscript; recruited patients and approved the final version of the manuscript. MK and MR recruited patients; analyzed and interpreted the data; reviewed the manuscript critically for important intellectual content. AF, SS, YSu, MU, NF, TKa, TU, RT, YS, TKo, HK, HM, TN, KK, and YT recruited patients and reviewed the manuscript critically for important intellectual content. YSh and MM contributed to interpretation of data and critically revised the manuscript. All authors approved the final version of the manuscript.

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