Comparison of three different exhaled nitric oxide analyzers in chronic respiratory disorders

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COMPARISON OF THREE DIFFERENT EXHALED NITRIC OXIDE ANALYZERS IN CHRONIC RESPIRATORY DISORDERS

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Running head: Exhaled nitric oxide and electrochemical analyzers.

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AM and SF contribute equally to the paper

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ABSTRACT

Background: Fractional exhaled nitric oxide (FeNO) measurement is a simple and non-invasive method for monitoring eosinophilic airway inflammation. New portable analyzers for FeNO measurements are constantly developed. The aim of our study was to evaluate the agreement of FeNO values measured by new portable analyzers.

Materials and methods: FeNO was measured in 20 healthy subjects, 20 asthmatic and 20 chronic obstructive pulmonary diseases patients by using the analyzers Niox-VERO, Vivatmo-PRO and HypAir-FeNO. Linear relationship was estimated with Pearson’s coefficient (r), absolute agreement by intra-class correlation coefficient (ICC) and bias with limits of agreement (95% of paired differences) were assessed according to Bland–Altman method.

Results: In the study population (58±14 years, 20 females), mean values of FeNO with their 95% confidence interval were 24.0 (18.6–29.4) with Niox-VERO, 19.6 (13.6–25.7) with Vivatmo-PRO and 20.4 (15.7–25.1) with HypAir-FeNO. FeNO measured with Niox-VERO was higher than Vivatmo-PRO (mean difference of paired values +4.3; limits -16.0 to 25.7 ppb) and HypAir-FeNO (+3.6; -12.2 to 19.4 ppb); Vivatmo-PRO and HypAir-FeNO showed large variability of paired differences (-0.7; -16.5 to 15.0 ppb). Measures correlated linearly with an imperfect absolute agreement: Niox-VERO vs. Vivatmo-PRO r=0.90 and ICC=0.87; Niox-VERO vs. HypAir-FeNO r=0.93 and ICC=0.90, Vivatmo-PRO vs. HypAir-FeNO r=0.96 and ICC=0.93.

Most of disagreement was greater in some asthmatic patients at high values of FeNO.

Conclusions: The present study indicates that absolute exhaled NO measurements may differ to a clinically relevant extent using Niox-VERO, Vivatmo-PRO and HypAir-FeNO. The devices cannot be used interchangeably.

Key words: Exhaled nitric oxide, COPD, asthma, respiratory, lung.
INTRODUCTION

Fractional exhaled nitric oxide (FeNO) has been proposed as a useful non-invasive marker of eosinophilic airway inflammation, and it could be a potential indirect predictor for response to steroid therapy [1]. Specific guidelines have been developed for standardized FeNO measurements, considering the chemiluminescence analysis the gold standard technique for its measurement [2]. Although the chemiluminescence analyzers used today perform excellently, they are somehow expensive, and require frequent calibrations and technical maintenance. Therefore, novel NO analyzers based on electrochemical sensor technology have been developed. Their main advantages are the portability, due to their smaller volume in comparison with the conventional stationary chemiluminescence analyzers, and the relative low cost [3].

Many analyzers used in the clinical practice have been tested in healthy subjects and in patients with airways disorders, showing a sufficient reproducibility and a good correlation with other techniques [4-8]. However, FeNO values obtained by using different devices may not compare [8], increasing the chance of false positives and false negatives [9].

Recently, new portable analyzers have been developed and commercialized, namely Vivatmo-PRO, Niox-VERO and HypAir-FeNO. To date, no data comparing measurements obtained with these devices are available. In this paper, we aimed at evaluating the agreement of FeNO measured by the three analyzers in healthy subjects (HS) and in patients with chronic respiratory disorders such as asthma and chronic obstructive pulmonary diseases (COPD).
SUBJECTS AND METHODS

Subjects
Sixty subjects were recruited, including 20 patients with asthma, 20 patients affected by COPD and 20 HS. The subjects’ characteristics are shown in Table I. The diagnosis of asthma and COPD was performed according to respectively the current GINA and GOLD guidelines.

The HS were recruited from hospital personnel and they had no history of asthma or any other chronic airway disease. The study was performed according to Good Clinical Practice standards and the Declaration of Helsinki and was approved by Maugeri Ethics Committee. The subjects gave written informed consent.

Exhaled NO measurements
FeNO was measured by using three different analyzers: Niox-VERO (Circeессia, Oxford UK), HypAir-FeNO (Medisoft, Dinant, Belgium) and Vivatmo-PRO (Bosch, Waiblingen, Germany).

Niox-VERO and Vivatmo-PRO did not require calibration. The HypAir-FeNO device was calibrated according to the standard procedure provided by the manufacturer. All analyzers were used according to the manufacturer's instructions. HypAir-FeNO worked in conjunction with a personal computer. The measurement ranges of the devices were 5–300 ppb for the Niox-VERO, 0–600 ppb for the HypAir-FeNO and 5–300 ppb for the Vivatmo-PRO.

Exhaled NO measurements were performed on all participants according to ATS/ERS guidelines [2]. Briefly, they were performed at a standardized exhalation flow rate of 50 ml/s.

To perform a valid exhalation maneuver, the flow parameters were controlled by both audio and visual feedback supplied by manufacturers, allowing the participant to maintain a constant exhaled breath flow rate.

FeNO measurements were obtained with each of the three analyzers in random order. Patients started with a single measurement on each analyzer, and repeated the measurements in the
same sequence for a total of two measurements on each device. All measurements were completed within 40 min on the same day.

Statistics

Data are presented as mean and standard deviation or 95% confidence interval (95% CI). Absolute frequency or percentage was reported for categorical data. Linear regression models were used to evaluate the relationship between variables. Coefficient of regression models (intercept and slope) were estimated with Pearson’s coefficient (r) for the evaluation of linear correlation and coefficient of determination ($R^2$) to measure the proportion of variability of the dependent variable attributable to the independent variable. By using a linear mixed regression model, the intra-class correlation coefficient (ICC) was estimated as measure of absolute agreement in FeNO values within subjects. The ICC is the ratio of variability between subjects to the total variability including subject variability and ranges from 0 to 1 (perfect agreement).

We evaluated the ICC within the same analyzer (two consecutive estimations of FeNO) and among Niox-VERO, Vivatmo-PRO and HypAir-FeNO (mean value of the first and second measurement). Bias among analyzers was assessed according to the Bland–Altman plot considering the difference between two paired measurements by their average. To derive an adjustment system among analyzers, an equations based on linear regression model (intercept+slope*FeNO) was derived from the first measurement and validated in the second evaluation.

A sample size of $15 \pm 2$ subjects was estimated for an ICCs of $\sim 0.90$ over two repeated measurements [10]. We enrolled a sample size of 20 subjects for each group, which estimated an ICCs $\sim 0.90$ with acceptable margins (wideness of 95% CI $\sim 0.15$).

The analyses were made using the STATA software, version 14 (StataCorp, College Station, TX, USA). A p value $< 0.05$ was considered statistically significant.
RESULTS

All patients were able to achieve technically acceptable maneuvers using all devices. Table I shows mean values with 95% CI of FeNO as average of repeated measurements with the same device. The value obtained by Niox-VERO at first and second measurements were, respectively, 23.7±20.5 and 24.3±21.8 with an intra-subject difference of -0.7±4.9 ppb. Similarly, consecutive values obtained by Vivatmo-PRO were 19.9±23.0 and 19.4±24.1 with a difference of 0.5±3.5 ppb. With HypAir-FeNO we obtained 20.2±17.7 and 20.6±19.0 with a difference of -0.3±3.4 ppb.

There was a strong absolute agreement between the first and second measurement for Niox-VERO. Table II shows ICCs for the absolute agreement within the same device in the overall population and in each subgroup. The ICCs were in the range 0.97-0.99 for the overall population, >0.95 in asthmatic patients and >0.90 in COPD. In healthy subjects, the agreement was lower for Niox-VERO than Vivatmo-PRO and HypAir-FeNO (ICC 0.81 vs. 0.97).

Comparison between Niox-VERO, Vivatmo-PRO and HypAir-FENO. Bland–Altman plots in Figure 1 shows high FeNO values obtained with Niox-VERO compared to Vivatmo-PRO and HypAir-FeNO, with a difference in some asthmatic patients at high values (panels A and B). The mean difference between Vivatmo-PRO and HypAir-FeNO was approximately zero with larger differences at greater eNO values (panel C). The overall mean of FeNO measured with Niox-VERO was higher than Vivatmo-PRO and HypAir-FeNO in the whole population and in all subgroups (Table I). The measures correlated (Niox-VERO and Vivatmo-PRO, r=0.90; Niox-VERO and HypAir-FeNO, r=0.93; Vivatmo-PRO and HypAir-FeNO, r=0.96; all p values <0.001), but the absolute agreement was not perfect. Table III shows ICCs for measures with different devices in the overall population and in each subgroup. The ICCs were ca. 0.90 in the overall population and in asthmatic patients for all comparisons, while the agreement was lower in COPD and healthy subjects, in particular for the device Niox-VERO. However, when the
agreement among devices was based on categories of FeNO (<25, 25-50 and >50 parts-per-billion; Table III), there was a high concordance in COPD and healthy subjects with a slight disagreement in asthmatic patients.

Adjustment between Niox-VERO, Vivatmo-PRO and HypAir-FENO

We used the linear relationships among FeNO values by different analyzers at the first measurement as adjustment system. Models had a high goodness of fit: the $R^2$ for Niox-VERO and Vivatmo-PRO was 0.793, for Niox-VERO and HypAir-FeNO was 0.857, for Vivatmo-PRO and HypAir-FeNO was 0.906. The second measurements was used to validate the equation. The first value measured with Niox-VERO was predicted by Vivatmo-PRO ($7.91+0.79*X$) or by HypAir-FeNO ($2.05+1.07*X$) with good adjustment over the second measurement (observed Niox-VERO 24.3±21.8, Vivatmo-PRO adjusted 23.3±19.1, and HypAir-FeNO adjusted 24.0±20.3 ppb). The first value measured with Vivatmo-PRO was predicted by Niox-VERO ($-3.80+1.00*X$) or by HypAir-FeNO ($-5.10+1.24*X$) with good correction over the second value (observed Vivatmo-PRO 19.4±24.1, adjusted Niox-VERO 20.6±21.8, and adjusted HypAir-FeNO 20.3±23.5 ppb). The first value measured with HypAir-FeNO was predicted by Niox-VERO ($1.25+0.80*X$) or by Vivatmo-PRO ($5.63+0.73*X$) with good adjustment over the second measure (observed HypAir-FeNO 20.6±19.0, adjusted Niox-VERO 20.8±17.5, and adjusted Vivatmo-PRO 19.9±17.7 ppb). For all predictions models, no significant recalibration was required by evaluating the linear regression of observed by predicted FeNO over the second measurement. The intercepts were not significantly different from zero and the slopes were not significantly different from the unity.
DISCUSSION

In this study, FeNO values obtained using three different NO analyzers were compared in an adult population of subjects with and without chronic respiratory disorders. We used the Niox-VERO, the Vivatmo-PRO and the HypAir-FeNO analyzers. FeNO values measured with the three devices showed a good correlation ($r^2$ always $> 0.9$), but only a moderate agreement. Furthermore, FeNO values measured in COPD patients with the Vivatmo-PRO were significantly lower than those measured with the Niox-VERO and HypAir-FeNO.

Several studies have evaluated the compatibility between devices using chemiluminescence analyzers and electrochemical sensors, or those equipped with electrochemical sensors [6, 11-18]. However, to our knowledge this is the first report on the relationship between exhaled NO values measured using three largely used analyzers.

There are several factors that may be responsible for the limited agreement of exhaled NO measurements, and some of them may be related to the method itself, including the sensor, the calibration procedures, the influence of ambient NO concentration, the different detection limit, and the airflow rate during the exhalation test [3].

The Vivatmo-PRO device has an infrared sensor while the other two are electrochemical devices, and such difference may affect the final result. In general, infrared sensors give a rapid response and may not require storage of the sample in a chamber, whereas electrochemical devices do require storage for the response.

The calibration protocol may also affect exhaled NO values. Niox-VERO and Vivatmo-PRO did not require calibration, while the HypAir-FeNO device explicitly required calibration as part of the regular maintenance protocol. The presence/absence of calibration procedure does not seem to be related to the device architecture as the infrared-sensor (Vivatmo-PRO) and one of the electrochemical (Niox-VERO) devices need calibration while the other electrochemical
(HypAir-FeNO) does not. Calibration could be linked to the narrower operational range of Niox-VERO and Vivatmo-PRO (5-300 ppb vs. 0–600 ppb for HypAir-FeNO).

The expiratory flow rate could also affect the measurement [19]. Although the same fixed expiratory flow rate (50 ml s\(^{-1}\)) was used for all devices, the exact flow rate is not under the operator control. The instruments allow for a 10% variability around a value of approximatively 50 ml/sec, therefore in the range 45 to 55 ml/sec.

Regarding the ambient NO, Niox-VERO and HypAir-FeNO, but not Vivatmo-PRO, present a charcoal filter to avoid inhalation of NO free air. If ambient NO interferes with measurements, increased values should always be observed for Vivatmo-PRO; however, since the Vivatmo-PRO measures values that well compare with the other analyzers, we considered the interference not relevant.

The different low-detection limit for exhaled NO (5 ppb for Niox-VERO and Vivatmo-PRO, and 0 ppb for HypAir-FeNO), as well as the measurement ranges (5-300 ppb for the Niox-VERO and the Vivatmo-PRO; 0–600 ppb for the HypAir-FeNO) might influence each other and eventually affect the final measurements. We are currently planning specific tests to verify how they both interfere with the measurements.

The differences in FeNO values obtained with different NO analyzers should be taken into consideration in the application and evaluation in clinical practice. In fact, the FeNO measurement may provide a sensitive and easy method for exploring airway inflammation [1]. Furthermore, it may represent an important biological marker in response to environmental modifications, with important implication in the screening of more frequent respiratory diseases including asthma, chronic cough and COPD [1, 20]. In the latter, the levels of FeNO are only occasionally higher but often similar or sometimes lower than healthy subjects probably because of the current or previous interference of cigarette smoking in COPD patients [21, 22].
Furthermore it has been suggested that FeNO measurement should be used also in the primary care setting as an alternative method to identify or rule out asthma, without referring to bronchial provocation, which may increase cost-effectiveness [3]. The use of FeNO as an adjunct to traditional asthma assessment tools (including spirometry, physical assessment, and symptom scores), would influence decision making in the care of patients with asthma in a specialty asthma setting with important clinical and cost-saving implications [23]. To this purpose, FeNO should be easily performed and devices used interchangeably. However, although a high correlation was observed, the Bland-Altman plot indicated a rather wide range of agreement limits.

Based on the Bland and Altman limits of agreement to assess the interchangeability of measurement methods [24], our results strongly suggest that the devices do agree with each other, but they should not be used interchangeably. These results were more evident in asthmatic patients for which we found the highest absolute deviations. Furthermore, if we consider the clinical relevant cut points for FeNO (<25, 25-50 and >50 ppb) [1], there was a high concordance in COPD and healthy subjects with a slight disagreement in asthmatic patients when using different devices (Table III). Thus, clinicians are free to measure FeNO level by any device, but they are recommended to always repeat the measurement in a patient with the same device.

In our study, a few limitations need to be considered when interpreting the findings. First, the lack of a reference standard value. In fact, we did not compare the analyzers to the gold standard chemiluminescence. However, the aim of our study was to compare the analyzers among them to know whether they can be used interchangeably within the same clinical service or patient group. Furthermore, several studies have compared a single electrochemical analyzer with the gold standard chemiluminescence [7, 12, 15-17], showing a relative good agreement. In fact, the FeNO values measured with the portable sensors presented variable values when compared...
to chemiluminescence, often showing higher values [8, 12, 16, 17] and sometime lower values [15].

Second, the level of patient training required for this study was greater than it is normally possible in daily clinical practice. This may explain why all patients were able to achieve technically acceptable maneuvers using each of the devices. Finally, patients under treatment including inhaled steroids were included in the study, and this may affect FeNO. However, the aim of our study was to evaluate the agreement among devices in real life and not to compare different diseases. We derived a correction equation to compare FeNO values among analyzers. The model was validated in a second consecutive measure available for subjects enrolled in the study. We are aware that our model is difficult to apply in clinical everyday practice; however, the simplicity of the adjustment factors allows for a useful easy correction procedure certainly applicable for research protocols. Further studies are needed to test the reliability of our proposed models in independent populations.

In conclusion, the present study indicates that absolute FeNO measurements may differ to a clinically relevant extent using Niox-VERO, Vivatmo-PRO and HypAir-FeNO, and that they cannot be used interchangeably. Further work is needed to ascertain whether the proposed correction factors can be applied to prevent systematic errors when the above devices are used in the daily clinical practice.
REFERENCES


Figure legend

Figure 1. Bland-Altman plot between Niox-VERO and Vivatmo-PRO (panel A), Niox-VERO and HypAir-FeNO (panel B), and Vivatmo-PRO and HypAir-FeNO (panel C). The dashed line indicates the mean of differences between the two-paired measurements, while the dotted lines indicate the limits of agreement that include 95% of all patients' difference data.
TABLE I. Subjects characteristics and exhaled nitric oxide concentrations measured by electrochemical devices.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Asthma</th>
<th>COPD</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=60</td>
<td>n=20</td>
<td>n=20</td>
<td>n=20</td>
</tr>
<tr>
<td>Female (n)</td>
<td>20</td>
<td>5</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Age(years)</td>
<td>58±14</td>
<td>55±10</td>
<td>70±9</td>
<td>45±10</td>
</tr>
<tr>
<td>Ex-smoker (n)</td>
<td>18</td>
<td>3</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Smoker (n)</td>
<td>16</td>
<td>3</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>FEV₁(L)</td>
<td>2.4±1.0</td>
<td>2.7±1.0</td>
<td>1.4±0.5</td>
<td>3.1±0.6</td>
</tr>
<tr>
<td>FEV₁(%)</td>
<td>83±25</td>
<td>87±25</td>
<td>62±16</td>
<td>106±6</td>
</tr>
<tr>
<td>FVC(L)</td>
<td>3.3±1.1</td>
<td>3.7±0.9</td>
<td>2.5±0.9</td>
<td>3.9±0.7</td>
</tr>
<tr>
<td>FVC(%)</td>
<td>96±21</td>
<td>99±17</td>
<td>83±22</td>
<td>112±6</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>70±15</td>
<td>72±16</td>
<td>59±12</td>
<td>80±3</td>
</tr>
<tr>
<td>FeNO (ppb)</td>
<td>24.0</td>
<td>44.0</td>
<td>14.9</td>
<td>13.1</td>
</tr>
<tr>
<td>Niox-VERO</td>
<td>(18.6–29.4)</td>
<td>(32.0–55.9)</td>
<td>(11.2–18.6)</td>
<td>(10.6–15.6)</td>
</tr>
<tr>
<td>Vivatmo-PRO</td>
<td>19.6</td>
<td>39.5</td>
<td>7.2</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td>(13.6–25.7)</td>
<td>(25.3–53.7)</td>
<td>(3.9–10.4)</td>
<td>(7.3–17.3)</td>
</tr>
<tr>
<td>HypAir-FeNO</td>
<td>20.4</td>
<td>37.8</td>
<td>10.4</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>(15.7–25.1)</td>
<td>(27.8–47.8)</td>
<td>(7.1–13.6)</td>
<td>(9.7–16.3)</td>
</tr>
</tbody>
</table>

Frequency, mean±standard deviation or mean (95% confidence interval). HS = healthy subjects; FEV₁ = forced expiratory volume at 1 sec; FVC = forced vital capacity; ppb = parts-per-billion.
**TABLE II.** Absolute agreement in exhaled nitric oxide values within subjects in two consecutive measures by the same device and in measures by different devices.

<table>
<thead>
<tr>
<th></th>
<th>Niox-VERO</th>
<th>Vivatmo-PRO</th>
<th>HypAir-FeNO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within the same device</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>All subjects</strong></td>
<td>0.973 (0.955-0.983)</td>
<td>0.989 (0.981-0.993)</td>
<td>0.983 (0.972-0.990)</td>
</tr>
<tr>
<td><strong>Asthma</strong></td>
<td>0.958 (0.903-0.982)</td>
<td>0.987 (0.969-0.995)</td>
<td>0.974 (0.940-0.989)</td>
</tr>
<tr>
<td><strong>COPD</strong></td>
<td>0.935 (0.852-0.973)</td>
<td>0.914 (0.808-0.964)</td>
<td>0.909 (0.799-0.962)</td>
</tr>
<tr>
<td><strong>HS</strong></td>
<td>0.809 (0.613-0.918)</td>
<td>0.971 (0.933-0.988)</td>
<td>0.971 (0.932-0.988)</td>
</tr>
<tr>
<td><strong>Between different devices</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Niox-VERO vs. Vivatmo-PRO</strong></td>
<td>0.873 (0.801-0.923)</td>
<td>0.900 (0.841-0.939)</td>
<td>0.926 (0.881-0.955)</td>
</tr>
<tr>
<td><strong>Asthma</strong></td>
<td>0.878 (0.737-0.948)</td>
<td>0.879 (0.740-0.949)</td>
<td>0.898 (0.778-0.957)</td>
</tr>
<tr>
<td><strong>COPD</strong></td>
<td>0.347 (0.088-0.744)</td>
<td>0.572 (0.286-0.817)</td>
<td>0.730 (0.489-0.884)</td>
</tr>
<tr>
<td><strong>HS</strong></td>
<td>0.235 (0.030-0.754)</td>
<td>0.299 (0.060-0.741)</td>
<td>0.780 (0.566-0.906)</td>
</tr>
</tbody>
</table>

Intraclass correlation coefficient (95% confidence interval). HS: healthy subjects.
TABLE III. Relative agreement in classifying exhaled nitric oxide values in the same category by different devices.

<table>
<thead>
<tr>
<th></th>
<th>Niox-VERO vs. Vivatmo-PRO</th>
<th>Niox-VERO vs. HypAir-FeNO</th>
<th>Vivatmo-PRO vs. HypAir-FeNO</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects, n=60</td>
<td>48 (80%)</td>
<td>55 (92%)</td>
<td>53 (88%)</td>
</tr>
<tr>
<td>Asthma, n=20</td>
<td>13 (65%)</td>
<td>18 (90%)</td>
<td>15 (75%)</td>
</tr>
<tr>
<td>COPD, n=20</td>
<td>18 (90%)</td>
<td>18 (90%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>HS, n=20</td>
<td>17 (85%)</td>
<td>19 (95%)</td>
<td>18 (90%)</td>
</tr>
</tbody>
</table>

Frequency and percentage. Categories of exhaled nitric oxide were <25, 25-50 and >50 parts-per-billion. HS: healthy subjects.
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