SHORT COMMUNICATION

Assessment of exhaled nitric oxide by a new hand-held device

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KEYWORDS
Exhaled nitric oxide; Logan; NIOX MINO; NObreath; Reproducibility

Summary
Background: Fractional exhaled nitric oxide (FENO) has been implicated as a pulmonary biomarker. The aim of this study was to compare the performance of a new hand-held device to a standard chemiluminescence analyzer and to another portable device.

Methods: FENO levels measured by NObreath (Bedfont) were compared to those of (1) a chemiluminescence detector (Logan, Logan Research) and (2) the electrochemical portable NIOX MINO (Aerocrine) in 18 healthy volunteers on three consecutive occasions: in the morning, 1 h and 24 h later.

Results: Comparing FENO levels obtained by NObreath to those by Logan values were similar and a very close linear relationship was found between the two devices ($r = 0.923$, $p < 0.001$). The mean inter-device difference in FENO level was $-3.45$ ppb and the limits of agreement (Bland–Altman test) were $-10.98$ and $4.08$ ppb. In the second series FENO levels obtained by NObreath were found to be slightly higher compared to those of NIOX MINO, but still showed a close correlation ($r = 0.681$, $p < 0.001$). The mean inter-device difference in FENO level was $4.36$ ppb and the limits of agreement were $-7.38$ and $16.1$ ppb. Analyzing the repeated FENO measurements, the mean coefficient of variation using NObreath tended to be lower than that of NIOX MINO ($11.8$ vs. $9.0\%$, $p = 0.342$), while it was similar as the value obtained with Logan ($16.9$ vs. $24.7\%$, $p = 0.059$), indicating that NObreath is suitable for use in clinical practice.

Conclusions: FENO values measured with NObreath are reproducible and in good agreement with those obtained by NIOX MINO and Logan indicating that NObreath is suitable for use in clinical practice.

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Introduction

Measurement of fractional exhaled nitric oxide (FENO) is a simple and completely non-invasive method for assessing airway inflammation. FENO has been implicated as a pulmonary biomarker in various respiratory diseases, particularly bronchial asthma. Chemiluminescence-based analysis is considered to be the gold standard technique for measuring FENO. Chemiluminescence analyzers are fast-responding, highly sensitive and specific for nitric oxide gas. However, several factors such as size, cost and frequent calibration limit their permeation into routine clinical practice.

To overcome these limitations electrochemical sensors suitable for FENO detection in exhaled breath have recently been developed, and incorporated into hand-held measuring devices. The first such portable device was the NIOX MINO (Aerocrine AB, Solna, Sweden). This instrument has been tested and was shown to provide values in good agreement with those obtained by the standard chemiluminescence analyzer.

More recently, a new hand-held device (NObreath; Bedfont Scientific Ltd., Rochester, Kent, UK) has been developed. The aim of this study was to compare the performance of the NObreath device with a standard, stationary chemiluminescence analyzer and the NIOX MINO.

Materials and methods

Study subjects

Thirty-six non-smoking healthy volunteers (16 men, 20 women, mean age: 46.6 ± 3.1 years) were enrolled into the study. Subjects had normal lung function values with no history of atopy, acute or chronic respiratory diseases in the previous four weeks. The research protocol was approved by the local Ethics Committee, and all subjects gave written informed consent to participate in the study.

Study design

Participants were divided into two groups (n = 18 for each group). In the first group FENO levels were measured by NObreath in parallel with Logan (study 1) and in the second with NIOX MINO (study 2) at three consecutive occasions: in the morning at 09:00 h (T1), 1 h (T2) and 24 h later (T3).

In study 1 measurements at each occasion were performed in triplicate and the mean value was used in the calculations. In study 2 only one measurement per device was performed at each occasion. Measurements were performed in randomized device order.

Measurements of fractional exhaled nitric oxide

FENO levels were recorded using either a chemiluminescence analyzer (Logan LR2500, Logan Research Ltd., Rochester, UK), the NIOX MINO or the NObreath at an exhalation flow rate of 50 mL/s. For NObreath, subjects first inhaled ambient air to near total lung capacity and then exhaled for 16 s at a constant flow rate through a mouthpiece into the device. The instrument has a color touch screen with visual prompts for subjects whilst taking the test. As a visual feedback, an eye level flow indicator (a small bullet in a plastic tube) helped the subjects keeping a constant flow during exhalation.

Statistical analysis

Data are presented as mean ± SEM or geometric mean with 95% CI when appropriate. For comparison between devices correlation coefficients were estimated and Bland–Altman plots were constructed. As FENO concentrations exhibited a non-normal distribution (Kolmogorov–Smirnov test), correlation coefficients were calculated by the Spearman method. Comparison between FENO levels of different devices was performed by the Mann Whitney test. The reproducibility of FENO readings was assessed by the coefficient of variation (CV) and the Bland–Altman test. All calculations were performed using GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA, USA) software package. A p value < 0.05 was considered significant.

Results

Study 1

In study 1 subject presented a FENO range of 3–49 ppb. Mean FENO levels (derived from triplicate measurements)

<table>
<thead>
<tr>
<th>Study</th>
<th>Logan (ppb)</th>
<th>NObreath (ppb)</th>
<th>NIOX MINO (ppb)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>T1</td>
<td>13.0 (10.1–16.7)</td>
<td>15.7 (11.7–21.9)</td>
<td>0.299a</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>13.5 (10.4–17.4)</td>
<td>14.8 (10.4–21.3)</td>
<td>0.351a</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>12.9 (9.9–16.6)</td>
<td>16.4 (12.3–21.9)</td>
<td>0.179a</td>
</tr>
<tr>
<td>Study 2</td>
<td>T1</td>
<td>14.9 (11.9–18.8)</td>
<td>12.6 (9.3–17.1)</td>
<td>0.409b</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>16.1 (13.3–19.6)</td>
<td>10.3 (7.8–13.4)</td>
<td>0.010b</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>16.3 (12.8–20.6)</td>
<td>10.9 (8.4–14.2)</td>
<td>0.043b</td>
</tr>
</tbody>
</table>

Data are presented as geometric mean with 95% CI.

a Logan vs. NObreath.
b NObreath vs. NIOX MINO, ppb: parts per billion.
using NObreath and Logan were similar (Table 1). A close linear relationship was found between the values obtained by the two devices at each assessment point (T1: $r = 0.897$, $p < 0.001$; T2: $r = 0.913$, $p < 0.001$; T3: $r = 0.938$, $p < 0.001$). Fig. 1a shows the correlation of FENO readings between the two devices.

The Bland–Altman plot showed a high degree of agreement between the two devices: the mean intrasubject difference in FENO level was $-3.95$ ppb and the limits of agreement were $-10.98$ and $4.08$ ppb (Fig. 2a).

The mean CV of repeated FENO readings were similar (NObreath: 11.8 vs. Logan: 9.0%, $p = 0.342$). The limits of agreement for repeated FENO measurements were $-4.6$ and $5.0$ for NObreath and $-3.0$ and $3.3$ ppb for Logan, respectively.

**Study 2**

In study 2 subjects presented a FENO range of 3–36 ppb. FENO readings by NObreath were higher compared to those of NIOX MINO; however, this only reached significance at assessment points T2 and T3 (Table 1). The mean difference in FENO level was 4.2 ppb. There was a close correlation between FENO levels measured by the two devices at each time-point (T1: $r = 0.662$, $p = 0.004$; T2: $r = 0.750$, $p < 0.001$; T3: $r = 0.654$, $p = 0.003$). Fig. 1b shows the correlation of FENO readings between the two devices.

Comparing the two devices, the mean intrasubject difference in FENO readings was $4.36$ ppb (Fig. 2b). On the Bland–Altman plot the limits of agreement were $-7.38$ and $16.1$ ppb.

The mean CV of repeated FENO readings using NObreath was lower compared to that of NIOX MINO; however, this difference was not significant (16.9 vs. 24.7%, $p = 0.059$). The limits of agreement for repeated FENO measurements were $-8.2$ and $6.5$ for NObreath and $-8.2$ and $13.2$ ppb for NIOX MINO, respectively.

**Discussion**

NObreath is new, portable, easy-to-use device with an electrochemical sensor similar to that of NIOX MINO, the other currently available hand-held device. Most of the technical parameters of the two instruments are similar.

In this study Logan and NObreath yielded similar FENO readings at each time-point. By contrast, FENO readings by NObreath were slightly higher (by approximately 4 ppb) than those of NIOX MINO at two of the three assessment points. Nevertheless, there was a close correlation between FENO values obtained by the devices in both parts of our study.

**Figure 1**

Correlation of fractional exhaled nitric oxide (FENO) readings between (a) NObreath and Logan and (b) NObreath and NIOX MINO (measurements at three time-points are presented in each graph). In panel (a) each point on the graph represents the mean value of three measurements, while in panel (b) each point corresponds to one measurement.

**Figure 2**

Agreement between (a) NObreath and Logan and (b) NObreath and NIOX MINO as reflected by the Bland–Altman plot comparing the inter-device mean to the inter-device difference (measurements at three time-points are presented in each graph). In panel (a) each point on the graph represents the mean value of three measurements, while in panel (b) each point corresponds to one measurement.
Repeated measurements were analyzed by the CV and the Bland–Altman method to determine the limits of agreement. Using NObreath we found that the mean CV tended to be lower and the limits of agreement were somewhat larger compared to NIOX MINO indicating that the reproducibility of FENO measurements with NObreath is slightly better. The CV for NIOX MINO was similar to previously reported values of our by other groups. By employing triplicate measurements at each assessment point in the first series, the mean CV of FENO readings by NObreath decreased from 16.9 to 11.8% and the limits of agreement for repeated measurements by NObreath also narrowed (−8.2 and 6.5 vs. −4.6 and 5.0). This finding is reasonable and indicates that the reproducibility of FENO measurements with NObreath can be improved with three parallel readings. Nevertheless, even when performing triplicate measurements, the reproducibility of FENO readings with Logan as assessed by the CV (9%) and the limits of agreement (−3.0 and 3.3 ppb) was still better compared to NObreath. Nonetheless, the difference was minor and from a clinical point of view we believe that this is negligible.

We are aware that our findings on healthy subjects cannot be automatically extrapolated to higher FENO ranges found typically in asthmatics. Nevertheless, FENO levels in our study were in a wide range, and from a clinical point of view, device accuracy is more important in a FENO range close to the cut-off between health and disease (20–35) ppb than at higher FENO levels. Finally, it is also possible that differences in FENO readings between NObreath and NIOX MINO may be augmented or minimized when FENO is measured in asthmatics.

In conclusion, FENO levels measured by a new portable device, the NObreath are reliable, reproducible and show a good agreement with those obtained by previously validated instruments. Our results indicate that NObreath is suitable for use in clinical practice.

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Conflict of interest

None of the authors have any conflict of interest to declare in relation to this work.

References