TECHNIQUES AND PROCEDURES

Measurement of Fraction of Exhaled Nitric Oxide With the Portable NIOX-MINO Monitor in Healthy Adults

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Measurement of the fraction of exhaled nitric oxide (FE\textsubscript{NO}) provides a non-invasive way to monitor asthma treatment in clinical practice. The aim of this study was to determine FE\textsubscript{NO} reference values for measurements recorded with the portable NIOX MINO monitor in a group of healthy volunteers. We also assessed the association between values recorded by the portable monitor and the N-6008 chemiluminescence analyzer used in our pulmonary function laboratory. The FE\textsubscript{NO} values obtained with the portable monitor were consistently higher than those recorded by the N-6008 monitor; the cutoff value for the portable monitor was 34 ppb (mean + 2SD). We detected a direct correlation (r=0.92) between the FE\textsubscript{NO} measurements recorded by the 2 monitors (P<0.001). The following equation expresses the relationship between measurements from the 2 devices: FE\textsubscript{NO}(NIOX-MINO) = 10 + [1.5 FE\textsubscript{NO}(N-6008)]. We did not observe statistically significant correlations between FE\textsubscript{NO} measurements and age, sex, body mass index, or spirometry.

Key words: Fraction of exhaled nitric oxide. Asthma. Inflammation.

Introduction

Asthma is a chronic inflammatory disease of the airways that leads to obstruction and bronchial hyperreactivity.\textsuperscript{1} In recent years various approaches to evaluating the degree of inflammation have been investigated with a view to assessing their utility for diagnosis and monitoring.\textsuperscript{2,3} One set of candidates proposed consists of markers of inflammatory activity, such as the eosinophil count in induced sputum and the fraction of exhaled nitric oxide (FE\textsubscript{NO}). We know that the synthesis of NO increases in asthma, induced by inflammatory cell activity, and that the increase can be detected in exhaled air. Studies have shown that an increase in FE\textsubscript{NO} provides a sensitive indicator of inflammation\textsuperscript{4,5} and in the proper context could offer an approach to diagnosing asthma, monitoring symptoms, and verifying compliance with drug therapy as well as adjusting corticosteroid dosages.\textsuperscript{6-8}

FENO is measured by chemiluminescence detection. As NO reacts with ozone it produces nitrogen dioxide and when the mixture is stable, light is emitted in proportion to the concentration of NO present in exhaled air. The online method is the one most often used and it is well established in the literature.\textsuperscript{9} Until recently, devices available to measure FENO have been complex, expensive, difficult to use with children, and suitable only for hospital use. Furthermore, FENO values reported in the literature have varied greatly from laboratory to laboratory. For these reasons, the NIOX-MINO (Aerocrine, Solna, Sweden) has entered the market. It offers a portable, simple, and economical way to monitor airway inflammation.

The aim of this study was to report useful information on the techniques and procedures for using the NIOX-MINO monitor in healthy adults.

Analysis of Exhaled Nitric Oxide

Determination of óxido nítrico en aire espirado (FE\textsubscript{NO}) mediante un equipo portátil (NIOX-MINO\textsuperscript{®} Aerocrine) en población sana

La determinación de óxido nítrico en aire espirado (FE\textsubscript{NO}) es una técnica no invasiva que se utiliza en la evaluación clínica y el tratamiento del asma. El objetivo de este estudio ha sido determinar los valores de referencia de FE\textsubscript{NO} en un grupo de voluntarios sanos mediante la utilización de un nuevo equipo portátil (NIOX-MINO\textsuperscript{®} Aerocrine), así como determinar el grado de relación que presenta con el equipo de determinación habitual en nuestro laboratorio de función pulmonar (sensor de quimioluminiscencia N-6008\textsuperscript{®} SIR). Según los resultados obtenidos, los valores de FE\textsubscript{NO} que da el equipo portátil son siempre superiores a los que ofrece el sensor habitual, con un valor de corte de 34 ppb (media + 2 desviación estándar). Existe una relación directa y significativa de la determinación de FE\textsubscript{NO} entre ambos equipos (r = 0.92; p = 0.001) con un factor de corrección de: FE\textsubscript{NO}(NIOX-MINO) = 10 + 1.5 FE\textsubscript{NO}(N-6008). La relación entre los valores de FE\textsubscript{NO} y la edad, el sexo, el índice de masa corporal y los valores espirométricos no fue estadísticamente significativa.

Palabras clave: Óxido nítrico en aire espirado. Asma. Inflamación.
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MINO device and to provide reference values in comparison with measurements from the conventional chemiluminescence analyzer used in our hospital.

Methods

Technical Description

FENO is usually measured in our laboratory with the N-6008 chemiluminescence sensor (SIR Madrid, Spain). In the online measurement technique recommended internationally,9 the patient starts from total lung capacity and exhales at a constant rate of 50 mL/s through a mouthpiece that provides a pressure of 5 to 20 cm H2O to ensure velum closure and prevent contamination from nasal NO. The mouthpiece is fitted with a filter that reduces the concentration of ambient NO. The output is a trace that allows an initial expiratory peak to be ignored so as to measure the plateau (≥3 seconds and ≤10% variability). Three exhalations must be performed, resulting in 3 NO plateau values according to international recommendations.9 The analyzer is precise to ±1% and readings have a margin for error between 0 and 300 parts per billion (ppb). The NO reading must be calibrated to 0 ppb before each test; circuits must be checked daily, the suction pump that samples air for NO analysis must be calibrated every 2 to 3 days, and carbon dioxide, flow, and volume analyzers must be calibrated every 3 to 4 months. Filters must be used and bacterial contamination checked.

The new NIOX-MINO device is a small, light, hand-held monitor measuring 24 × 13 × 10 cm and weighing 800 g. It is equipped with a sensor that detects NO by electrochemical reaction rather than by conventional chemiluminescence analysis. A main difference between the new and the traditional technique is that the patient inhales filtered air through the monitor until reaching total lung capacity, for the chemiluminescence analyzer, on the other hand, ambient air is inhaled. Next the patient exhales through the device at a flow of 50 mL/s, guided by a light and sound signal to guide and assure a steady flow. A single measurement is recommended by the manufacturer. The result is expressed on a digital display and a patient’s FENO level at different times can be saved in a memory card. The device had a lower limit of precision of 3% in measurements less than 30 ppb, of less than 10% for values over 30 ppb, and a reading has a lower limit of precision of 3% in measurements less than 30 ppb, of less than 10% for values over 30 ppb, and a reading range between 5 and 300 ppb. Calibration is unnecessary (Figure 1).

Volunteers

Twenty-eight healthy non-smoker volunteers (7 males, 21 females) were chosen. All had spirometry within normal reference values, had no concurrent illnesses, and were following no regular treatments. The characteristics of the group are shown in the table.

Each volunteer began FENO measurement in random order with either the conventional chemiluminescence analyzer (N-6008), carrying out 3 maneuvers with the online technique,9 or the NIOX-MINO monitor, performing a single maneuver according to the manufacturer’s instructions. All measurements were always made at the same time of day, approximately 2 hours after a meal. Finally, all volunteers underwent spirometry.

Statistical Analysis

FENO findings were expressed as means (SD) and compared with the Mann–Whitney test. Individual results were compared with the Pearson correlation coefficient. All comparisons were bilateral and the usual level of significance of 5% (α=0.05) was used. SPSS version 11.5 software was used to carry out the analysis.

Results

The mean FENO reading was 20 (7) ppb (range, 8-41 ppb) with the NIOX-MINO monitor and 7 (5) ppb (range, 1-19 ppb) with the N-6008 analyzer. According to previously published NO reference values for the chemiluminescence analyzer, an abnormal FENO level was set as 20 ppb or more9 (Figure 2). A statistically significant direct correlation between FENO measurements from the 2 devices was detected (r=0.92; P<.001). FENO readings from the NIOX-MINO device were always higher than those obtained with the N-6008. The correction indicated by linear correlation is represented by the equation FENO (NIOX-MINO) = 10 + [1.5 × FENO (N-6008)] (Figure 3). No statistically significant correlation was found between FENO values and age, sex, body mass index, or spirometry (P>.05).

Characteristics of the Study Population (n=28)*

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<table>
<thead>
<tr>
<th>Sex</th>
<th>Age, y</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>Body mass index, kg/cm²</th>
<th>FEV1, % reference</th>
<th>FVC, % reference</th>
<th>FEV1/FVC, % reference</th>
<th>PEF, % reference</th>
<th>PEF/FVC, % reference</th>
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<tr>
<td>Men</td>
<td>21</td>
<td>163.85</td>
<td>67.43</td>
<td>21</td>
<td>103.57 (10.02)</td>
<td>137.36 (20.63)</td>
<td>83.32 (11.8)</td>
<td>83.32 (11.8)</td>
<td>83.32 (11.8)</td>
</tr>
<tr>
<td>Women</td>
<td>21</td>
<td>163.85</td>
<td>67.43</td>
<td>21</td>
<td>103.57 (10.02)</td>
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| Data are mean (SD) or number of individuals. FEV1 indicates forced expiratory volume in 1 second; FVC, forced vital capacity. |

**Figure 1. The NIOX-MINO monitor (Aerocrine, Solna, Sweden) for measuring fraction of exhaled nitric oxide.**
Discussion

The measurements obtained with the NIOX-MINO monitor in our clinical practice are slightly higher than those obtained with the N-6008 analyzer, a pattern that can be seen in the distribution of readings in Figure 2 (mean higher reading of 14 ppb).

There is very good correlation between FE NO measurements recorded by the 2 devices, with a correction of \( y = 10 + 1.5x \) for each FE NO measurement obtained with the N-6008 (Figures 2 and 3), consistent with the findings of other authors.\(^{11-13}\)

The cutoff value (mean + 2 SD) of 17 ppb for the N-6008 analyzer was within the reference values reported by other laboratories and recommended in international guidelines.\(^9,11\) Likewise, the cutoff of 34 ppb for the NIOX MINO was consistent with reference values reported by other authors.\(^{12,13}\)

FENO measurement with the new NIOX MINO device is unaffected by individual anthropometric or spirometry differences, as no significant differences were found in relation to age, sex, body mass index, or spirometry, as described by other authors.\(^{11-13}\)

An advantage of the NIOX-MINO over the chemiluminescence analyzer is that there is less risk of breath being contaminated by ambient NO. The patient inhales through the MIOX-MINO, thus ensuring that air free of NO is taken in. In contrast, the patient inhales ambient air for the chemiluminescence analyzer. FE\(_{NO}\) can be used as a marker of inflammation in patients with inflammatory respiratory diseases like asthma, provided FE\(_{NO}\) reference values are established for each laboratory. In this way, it is possible to obtain simple, fast and reproducible measurements for guiding better treatment of the patient. FE\(_{NO}\) is useful for clinical monitoring of treatment effectiveness and compliance. With the introduction of these new devices in the near future it will be possible to use FE\(_{NO}\) measurements to monitor asthma in the home.

A limitation of the NIOX-MINO monitor is that it does not produce a trace of the FE NO curve to allow identification of a plateau corresponding to the bronchial fraction of NO (whereas the chemiluminescence analyzer does). Rather, the hand-held monitor issues a digital reading of the FENO value. As a result, the observer cannot adjust the output, possibly affecting the reproducibility of the technique. However, taking only a single measurement is advised. Other limitations derive from the respiratory maneuvers required and the possible lack of cooperation. Contraindications have not been described.

The NIOX-MINO is able to carry out 1500 measurements, each of which would cost €12. For use of the device to be cost-effective and comparable to the chemiluminescence analyzer, the manufacturer recommends performing 3000 measurements per year.

In summary, FE NO measurement is a noninvasive, rapid and harmless technique that provides an inflammatory marker for studying respiratory diseases such as asthma. In the clinical setting it can allow underlying inflammation to be quantified for monitoring the patient’s condition, adjusting dosages, or verifying therapeutic compliance. It can also provide an approach...
to asthma diagnosis based on inflammation. Each laboratory requires reference values with its own equipment to better interpret data for this type of biological measurement. We can expect that hand-held equipment such as the NIOX-MINO described will be used in the future not only in hospital settings but also in outpatient clinics, homes, or in large-scale epidemiologic studies.

REFERENCES