Comparison of Exhaled Nitric Oxide Measurement With Conventional Tests in Steroid-Naive Asthma Patients

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Abstract. Background: Nitric oxide (NO) is a molecule with potent biological activity that plays an important role in the physiology of the respiratory system. Increased expression of inducible nitric oxide synthase (iNOS) and elevated fractional concentration of exhaled nitric oxide (FENO) are seen in asthmatic patients. Measurement of FENO has become increasingly recognized for use in the evaluation of bronchial inflammation during monitoring of antiinflammatory treatment.

Objectives: The aim of this study was to evaluate FENO in a group of steroid-naive asthmatics and assess the relationship of this parameter with the results of other tests used in the diagnosis of asthma and monitoring of antiinflammatory treatment in asthmatic patients.

Methods: The study was conducted in a group of 101 steroid-naive asthmatics (56 allergic and 45 nonallergic) and 39 healthy volunteers. All patients underwent measurement of FENO, skin prick tests with common inhaled allergens, analysis of serum eosinophil cationic protein (ECP) and blood eosinophilia, and flow-volume spirometry. When the forced expiratory volume in the first second (FEV1) was less than 80% of predicted, reversibility of airway obstruction with a ß2-agonist was assessed. A nonspecific bronchial provocation test with histamine was carried out in asthmatic patients with a baseline FEV1 of more than 70% of predicted.

Results: Compared to the healthy volunteers, FENO was elevated in both groups of asthmatics. FENO in the allergic asthma group was higher than in the group of nonallergic asthmatics. In allergic and nonallergic asthmatics, FENO was significantly correlated with bronchial hyperresponsiveness to histamine, reversibility of airway obstruction, serum ECP levels, and blood eosinophilia. FENO did not correlate with baseline FEV1 in either group of asthmatics. In 31% of nonallergic and 9% of allergic patients, FENO was less than 20 parts per billion.

Conclusions: We suggest that measurement of FENO could be clinically useful in steroid-naive asthmatics and should be more widely used in clinical practice. Measurement of FENO is a noninvasive, simple, and reproducible procedure, the results of which correlate with other routinely used methods in the diagnosis of asthma. However, it is worth noting that some patients, especially those with nonallergic asthma, do not display elevated FENO.

Key words: Asthma. Exhaled nitric oxide. Pulmonary function tests. Eosinophil cationic protein. Blood eosinophilia. IgE.

Resumen. Antecedentes: El óxido nítrico (NO) es una molécula con potente actividad biológica que juega un importante papel en la fisiología del sistema respiratorio. En los pacientes asmáticos se observa un incremento de la expresión de la óxido nítrico sintetasa inducible (iNOS), así como una concentración fraccional elevada de óxido nítrico espirado (FENO). Ha aumentado el reconocimiento de la medición del FENO como instrumento de evaluación de la inflamación bronquial durante la monitorización del tratamiento antiinflamatorio.

Objetivos: El propósito del estudio fue evaluar el FENO en un grupo de asmáticos sin tratamiento previo con esteroides y su relación con otras pruebas utilizadas en el diagnóstico del asma y la monitorización del tratamiento antiinflamatorio en pacientes asmáticos.

Métodos: En el estudio participaron 101 asmáticos sin tratamiento previo con esteroides (56 alérgicos y 45 no
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Introduction

Nitric oxide (NO) is a molecule with potent biological activity that plays an important role in the physiology of the respiratory system. It is synthesized endogenously from L-arginine in a reaction catalyzed by nitric oxide synthase (NOS). Two isoforms of NOS—endothelial and neuronal—are involved in the regulation of respiratory system functions, while a third, inducible form (iNOS) is involved in inflammation and response to infections [1].

Increased expression of iNOS and elevated fractional concentration of exhaled nitric oxide (FENO) are seen in asthmatic patients [2]. Proinflammatory cytokines, bacterial lipopolysaccharides, allergen exposure, and air pollutants cause increased expression of iNOS [3]. Recently, measurement of the fractional concentration of exhaled nitric oxide (FENO) has become increasingly recognized for use in the evaluation of bronchial inflammation during monitoring of antiinflammatory treatment [4, 5].

Markers of airway inflammation, such as eosinophils from induced sputum and airway biopsy specimens, are elevated in patients with asthma [6] and have been found to correlate with FENO or bronchial hyperreactivity in patients not treated with inhaled corticosteroids [7, 8]. Airway responsiveness is known to be related to baseline lung function [9]. However, these findings are not supported by all studies and some authors have reported that there were no correlations between bronchial hyperresponsiveness, lung function, and markers of airway inflammation [10]. Recently Smith et al [11] reported that FENO measurements and induced sputum analysis are more valuable methods than conventional tests for the diagnosis of asthma.

The aim of this study was to evaluate FENO in steroid-naive asthmatic patients and to assess possible correlations between these measurements and the results of tests used in routine diagnosis of asthma (baseline lung function, reversibility of airway obstruction, and evaluation of nonspecific bronchial hyperreactivity) and other laboratory tests commonly associated with asthma, such as serum concentration of IgE, eosinophil cationic protein (ECP), and peripheral blood eosinophilia.

Methods

Patients

The study involved a group of 101 steroid-naive patients with mild to moderate asthma (56 allergic and 45 nonallergic). Asthma was diagnosed according to the criteria recommended by the Global Initiative for Asthma [12]. All patients were in a stable condition free from acute exacerbations and respiratory tract infections during the previous 2 months. Patients who presented other factors that could alter FENO—such as smoking and nitrate–rich diet, but not asthma, features of atopy, or allergic rhinitis—were excluded from the study. Prior to the beginning of the study, patients were allowed to take short- and long-acting β2-agonists. Asthmatic patients who had been treated with inhaled steroids in the past were excluded from the study. FENO measurement, skin prick tests with commonly encountered Aeroallergens (house dust mites, trees, weeds, grasses, cat, Alternaria, and Cladosporium), and flow-volume spirometry were performed in each asthmatic patient at the beginning of the study. When the baseline forced expiratory volume in the first second (FEV1) was less than 80% of predicted, reversibility with an inhaled β2-agonist (400 µg of salbutamol) was assessed. A histamine challenge was carried out in patients with baseline FEV1 more than 70% of predicted. In the allergic asthma group, 37 patients had a history of perennial allergic rhinitis for at least 12 months. Allergic rhinitis
was diagnosed based on history of symptoms and results of skin prick tests.

Thirty-nine healthy volunteers were used as a negative control group. All of them underwent analysis of \( FE_{NO} \), flow-volume spirometry, and skin prick tests with common aeroallergens. They had an \( FEV_1 \) greater than 80% of predicted. They were free of respiratory tract infection for 2 months prior to the study and from other significant illnesses known to affect \( FE_{NO} \) measurements (smoking, nitrate-rich diet, allergic rhinitis).

Peripheral blood eosinophils, total serum IgE, and ECP were analyzed in blood samples from all asthmatic patients and healthy volunteers.

Asthmatic patients and healthy volunteers were nonsmokers and had not been passive smokers in the previous year.

The study protocol was approved by the research ethics committee of the Medical University of Bialystok (reference R-I-003/49/2002 and R-I-003/187/2003). Informed consent was obtained from all patients enrolled in the study.

### Measurements

\( FE_{NO} \) was measured in all of the asthmatic patients and healthy subjects using the chemiluminescence technique with a Sievers 280i NO Analyzer (Boulder, Colorado, USA). The measurements were performed at an expiratory flow of 50 mL/s [13]. The duration of exhalation had to be at least 6 seconds to produce a stable NO level for 3 seconds. Three recorded \( FE_{NO} \) measurements were obtained for each subject. Repeat measurements were performed until the 3 values agreed to within 10% of the mean. The mean value of the 3 measurements was recorded as the final \( FE_{NO} \).

Baseline spirometry was performed using a MasterScreen Pneumo PC spirometer (Jaeger, Hoechberg, Germany). Spirometry was performed according to American Thoracic Society guidelines [14]. Patients had to refrain from use of inhaled bronchodilators for at least 6 and 12 hours for short- and long-acting \( \beta_2 \)-agonists, respectively. When the baseline \( FEV_1 \) value was lower than 80% of predicted, a test of airway obstruction reversibility was performed in which spirometry was repeated 15 minutes after inhalation of 400 \( \mu \)g of salbutamol using a spacer device (Volumatic, Brentford, UK). In patients with a baseline \( FEV_1 \) value higher than 70% of predicted, histamine challenge was performed as previously described [15,16].

Blood eosinophil count was measured using a hematologic analyzer (Coulter Electronic GmbH, Miami, Florida, USA). Total serum IgE concentration and serum ECP concentration were measured by immunoassay using the ImmunoCAP system (Pharmacia Diagnostics, Uppsala, Sweden).

### Statistical Analysis

Because of the lack of normal distribution in some variables, statistical analyses were performed using nonparametric tests. Comparisons between groups were performed with the Wilcoxon test for paired samples. All values were expressed as means \( \pm \) SD. The relationship between studied parameters was assessed using Pearson’s linear correlation coefficient. \( P \) values of less than .05 were considered statistically significant.

### Results

The characteristics of the patients and healthy volunteers are presented in Table 1.

**Table 1. Characteristics of Study Subjects and Healthy Volunteers**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Allergic Asthma</th>
<th>Nonallergic Asthma</th>
<th>( P ) (Allergic vs Nonallergic Asthma)</th>
<th>Healthy Volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>56</td>
<td>45</td>
<td>–</td>
<td>39</td>
</tr>
<tr>
<td>Asthma severity, mild/moderate, ratio</td>
<td>42/14</td>
<td>29/16</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sex, F/M, ratio</td>
<td>31/25</td>
<td>28/17</td>
<td>–</td>
<td>24/15</td>
</tr>
<tr>
<td>Age, y</td>
<td>32±12</td>
<td>40±12</td>
<td>&gt;.05</td>
<td>33.5±15.2</td>
</tr>
<tr>
<td>Duration of symptoms, y</td>
<td>6.2±4.3</td>
<td>4.9±4.2</td>
<td>&gt;.05</td>
<td>–</td>
</tr>
<tr>
<td>Baseline ( FEV_1 ), % predicted</td>
<td>87.8±16.7</td>
<td>82.6±12.2</td>
<td>.09</td>
<td>101±4.8†,‡</td>
</tr>
<tr>
<td>( \Delta FEV_1 ), %</td>
<td>27.2±11.6</td>
<td>20.2±7.1</td>
<td>.02</td>
<td>–</td>
</tr>
<tr>
<td>PC20histamine ( \text{FEV}_1 ), mg/mL</td>
<td>2.0±1.93</td>
<td>2.8±1.96</td>
<td>.07</td>
<td>–</td>
</tr>
<tr>
<td>Blood eosinophil count, cells/mm(^3)</td>
<td>246.6±105.3</td>
<td>211±97.8</td>
<td>.06</td>
<td>119.2±36.5†,‡</td>
</tr>
<tr>
<td>Serum ECP, ( \mu )g/L</td>
<td>15.1±6.81</td>
<td>11.8±5.72</td>
<td>.09</td>
<td>4.38±4.7†,‡</td>
</tr>
<tr>
<td>Total Serum IgE, kU/L</td>
<td>212±145</td>
<td>78±69</td>
<td>.004</td>
<td>65±51‡</td>
</tr>
<tr>
<td>BPT</td>
<td>49</td>
<td>37</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Reversibility test</td>
<td>19</td>
<td>20</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Data are shown as means \( \pm \) SD, unless otherwise indicated. \( FEV_1 \) indicates forced expiratory volume in the first second; \( \Delta FEV_1 \), increase in \( FEV_1 \) after inhaling 400 \( \mu \)g salbutamol; PC20histamine \( \text{FEV}_1 \), provocative concentration of histamine that caused a 20% reduction in \( FEV_1 \); ECP, eosinophil cationic protein; BPT, bronchial provocation test with histamine; † Values significantly different from allergic asthma, \( P < .05 \); ‡ Values significantly different from nonallergic asthma, \( P < .05 \)
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Table 2. Correlations Between Serum ECP and FENO or Results of Pulmonary Function Tests

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>FENO</th>
<th>FEV₁</th>
<th>PC20 Histamine FEV₁</th>
<th>ΔFEV₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic asthma</td>
<td>r = 0.57</td>
<td>P = .0005</td>
<td>r = -0.37</td>
<td>P = .007</td>
</tr>
<tr>
<td>Nonallergic asthma</td>
<td>r = 0.47</td>
<td>P = .001</td>
<td>r = -0.45</td>
<td>P = .005</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>r = -0.06</td>
<td>P = .7</td>
<td>r = -0.02</td>
<td>P = .89</td>
</tr>
</tbody>
</table>

*FENO indicates fractional concentration of exhaled nitric oxide; ECP, eosinophil cationic protein; FEV₁, forced expiratory volume in the first second; ΔFEV₁, increase in FEV₁ after inhaling 400 µg salbutamol; PC20histamine FEV₁, provocative concentration of histamine that caused a 20% reduction in FEV₁.*

Table 3. Correlations Between Blood Eosinophil Count and FENO or Results of Pulmonary Function Tests

<table>
<thead>
<tr>
<th>Study Groups</th>
<th>FENO</th>
<th>FEV₁</th>
<th>PC20 Histamine FEV₁</th>
<th>ΔFEV₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic asthma</td>
<td>r = 0.69</td>
<td>P = .0002</td>
<td>r = -0.41</td>
<td>P = .003</td>
</tr>
<tr>
<td>Nonallergic asthma</td>
<td>r = 0.64</td>
<td>P = .0001</td>
<td>r = -0.42</td>
<td>P = .008</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>r = -0.05</td>
<td>P = .72</td>
<td>r = -0.16</td>
<td>P = .3</td>
</tr>
</tbody>
</table>

*FENO indicates fractional concentration of exhaled nitric oxide; FEV₁, forced expiratory volume in the first second; ΔFEV₁, increase in FEV₁ after inhaling 400 µg salbutamol; PC20histamine FEV₁, provocative concentration of histamine that caused a 20% reduction in FEV₁.*

significantly higher in both groups of asthmatics compared with healthy volunteers (P < .05). Comparing both groups of asthmatics, higher ECP concentrations and blood eosinophil counts were obtained in patients with allergic asthma. However, these differences were not statistically significant. There was a significant positive correlation between FENO levels and serum ECP (Table 2) and between FENO levels and blood eosinophil count (Table 3). Also, there was a significant negative correlation between the provocative concentration of histamine causing a 20% reduction in FEV₁ (PC20FEV₁) and both serum ECP (Table 2) and blood eosinophil count (Table 3).

The FENO observed in the allergic asthma group was significantly higher than in patients with nonallergic asthma (84.0 ± 51.4 parts per billion [ppb] vs 45.8 ± 32.6 ppb; range, 10-210 ppb and 12-116 ppb, respectively; P = .0001).

Compared to the healthy control group, the FENO in both groups of asthma patients was significantly elevated (12.9 ± 4.6 ppb; range, 7.8-29 ppb; P < .0001) (Figure 1).

In the allergic asthma group, significantly higher FENO values were found in patients with moderate asthma than in those with mild asthma (109.0 ± 56.94 ppb vs 75.66 ± 47.27 ppb; P = .03), while in patients with nonallergic asthma the differences were not statistically significant (54.18 ± 36.87 ppb vs 47.20 ± 31.35 ppb; P = .5). Moreover, in the allergic asthma group, FENO

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Figure 1. Exhaled NO levels in the groups of asthma patients and healthy volunteers. FENO indicates fractional concentration of exhaled nitric oxide; ppb, parts per billion.
values were significantly higher in patients with allergic rhinitis (37 patients) compared with those without (19 patients) (93.4±46.2 ppb vs 75.5±51.1 ppb; P = .02).

FENO values were within the predefined normal range of up to 20 ppb [11] in only 5 (9%) and 14 (31%) patients in the allergic and nonallergic groups, respectively (Figure 2A and B). There was no correlation between FENO and the time-course of disease symptoms or total serum IgE in any of the groups studied. Also, there was no correlation between FENO and baseline FEV1 in the group of allergic (r = 0.02; P = .87) and nonallergic asthma patients (r = −0.22; P = .13) (Figure 3).

In both groups of asthma patients, FENO displayed a significant negative correlation with the PC20FEV1 for histamine (allergic asthma, r = −0.62, P = .00002; nonallergic asthma, r = −0.41, P = .01; Figure 4).

FENO levels displayed a significant correlation with the reversibility of airway obstruction after β2-agonist inhalation in both groups of patients (allergic asthma, r = 0.51, P = .02; nonallergic asthma, r = 0.47, P = .03; Figure 5).
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Figure 4. Relationship between $F_{ENO}$ and PC20histamine FEV$_1$ in both groups of asthma patients. Significant correlations were observed for both allergic asthma ($r = -0.6218, P = .00002$) and nonallergic asthma ($r = -0.4101, P = .01$). $F_{ENO}$ indicates fractional concentration of exhaled nitric oxide; ppb, parts per billion; PC20histamine FEV$_1$, provocative concentration of histamine that caused a 20% reduction in FEV$_1$.

Figure 5. Correlations between $F_{ENO}$ and reversibility of airway obstruction following administration of 400 µg of salbutamol. Significant correlations were observed for both allergic asthma ($r = 0.51061, P = .02$) and nonallergic asthma ($r = 0.47241, P = .03$). $F_{ENO}$ indicates fractional concentration of exhaled nitric oxide; ppb, parts per billion; FEV$_1$, forced expiratory volume in the first second.
Discussion

In patients with asthma, elevated FENO values are thought to arise from increased expression and activity of iNOS in airway epithelial and inflammatory cells [8, 17]. The increased production of NO in asthma may have an amplifying effect on airway inflammation. The crucial point for evaluating the usefulness of measuring FENO was to identify possible correlations between this variable and the results of other recognized tests used in the assessment of allergic inflammation in asthmatic patients.

It has been reported that FENO correlates well with conventional markers of airway inflammation. FENO correlates with the results from examinations of bronchial biopsies and bronchoalveolar lavage (BAL) [18-20]. Compared with procedures such as BAL and airway biopsy, measurement of NO is noninvasive, safe, and causes no inconvenience to the patient. Studies by Jatakanon et al [8, 21] demonstrated a relationship between exhaled NO and sputum eosinophils. Other studies, performed by Piacentini et al [22] in steroid-naive patients and Mattes et al [23] in children with corticosteroid-dependent asthma, have drawn similar conclusions. A statistically significant correlation between FENO and both the total number of blood eosinophils and the percentage of blood eosinophils in allergic asthma has been demonstrated by Silvestri et al [24].

The results of this study indicate a statistically significant correlation between FENO and serum ECP or blood eosinophil count and between bronchial hyperreactivity and those serum markers in both groups of asthma patients. There was no correlation between serum markers and FEV1 or the reversibility of airway obstruction.

The use of FENO measurements in diagnosis and treatment monitoring of asthmatic patients depends on the correct interpretation of the results. There are several factors that determine FENO and there is a relatively large variation in FENO between individuals. This will necessitate the establishment of stable baseline values in individual patients as a basis for comparison.

Our results showed significantly higher FENO values in patients with allergic asthma than in those with nonallergic asthma. Individuals with a diagnosis of perennial allergic rhinitis in addition to asthma had significantly higher FENO than individuals with asthma alone.

The results of FENO measurements allow assessment of the clinical usefulness of this test and suggest that it is more useful in allergic asthmatics. Predefined normal values for FENO (< 20 ppb) were found more often in the group of patients with nonallergic asthma. The results obtained in this study confirm previous suggestions that measurement of FENO is most valuable at the time of diagnosis of asthma and then in the monitoring of antiinflammatory treatment and the course of the disease [11].

The presence and severity of bronchial hyperreactiveness has been correlated with the activation of inflammatory cells [25]. The assessment of nonspecific bronchial hyperreactivity is a very useful test if it is performed to confirm the diagnosis of asthma or monitor antiinflammatory treatment [26]. Al-Ali et al [27] demonstrated a correlation between increased FENO and both nonspecific bronchial hyperreactivity to histamine and the variability of daily peak flow. Similar correlations have been demonstrated by Dupont et al [28], who used methacholine as the bronchoconstrictor in steroid-naive patients.

Our study showed that FENO correlates well with the degree of bronchial hyperresponsiveness to histamine in a steroid-naive population of patients with allergic and nonallergic asthma. The correlation was stronger in the allergic asthma group. The reported correlation of FENO with bronchial hyperresponsiveness suggests that this marker could prove to be useful as a screening tool for asthma [28].

Although it is increasingly recognized that pulmonary function tests do not reflect airway inflammation, they continue to represent the standard method for assessing asthma. FEV1 is the most commonly used parameter in diagnosis and treatment monitoring of asthmatic patients. Some authors are doubtful about the usefulness of FEV1 assessment, especially in subjects with mild asthma [11]. In general, FENO does not correlate with lung function parameters in stable asthma patients. FENO responds faster than spirometry results to changes affecting airway inflammation [29]. In some studies, the correlation between the reversibility of airway obstruction and exhaled nitric oxide levels in children with stable asthma has been described [30]. Our results did not reveal any correlation between FENO and FEV1. It is worth noting that in some patients, values for spirometric indices were almost normal. In both groups there was a statistically significant correlation between FENO and reversibility of airway obstruction.

Conclusions

Assessment of FENO has attracted increasing interest for use in diagnosis and treatment monitoring in asthmatic patients. Steroid-naive asthma patients, especially those with allergic asthma, present high FENO values. FENO correlates with the results of other tests used in the diagnosis of asthma (assessment of bronchial hyperreactivity and reversibility of airway obstruction). FENO is not correlated with baseline FEV1; this observation is very important in patients with mild asthma, since spirometric indices are not very useful in some such patients. Measurement of FENO is a rapid, simple, reproducible test, and it can be performed in all patients in whom spirometry can be carried out. High values for FENO and patient symptoms suggestive of the presence of asthma can confirm the diagnosis of this disease. On the other hand, normal levels of exhaled NO, especially in patients with no atopic symptoms, do not exclude asthma. More studies are needed to confirm the usefulness of measuring exhaled NO in clinical practice.
References


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