Original Article

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 (F_{ENO}) are seen in asthmatic patients. Measurement of F_{ENO} 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 F_{ENO} 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 F_{ENO}, 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 (FEV_1) was less than 80% of predicted, reversibility of airway obstruction with a ß$_2$-agonist was assessed. A nonspecific bronchial provocation test with histamine was carried out in asthmatic patients with a baseline FEV_1 of more than 70% of predicted.
Results: Compared to the healthy volunteers, F_{ENO} was elevated in both groups of asthmatics. F_{ENO} in the allergic asthma group was higher than in the group of nonallergic asthmatics. In allergic and nonallergic asthmatics, F_{ENO} was significantly correlated with bronchial hyperresponsiveness to histamine, reversibility of airway obstruction, serum ECP levels, and blood eosinophilia. F_{ENO} did not correlate with baseline FEV_1 in either group of asthmatics. In 31% of nonallergic and 9% of allergic patients, F_{ENO} was less than 20 parts per billion.
Conclusions: We suggest that measurement of F_{ENO} could be clinically useful in steroid-naive asthmatics and should be more widely used in clinical practice. Measurement of F_{ENO} 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 $F_{ENO.}$

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 (F_{ENO}). Ha aumentado el reconocimiento de la medición del F_{ENO} como instrumento de evaluación de la inflamación bronquial durante la monitorización del tratamiento antiinflamatorio.
Objetivos: El propósito del estudio fue evaluar el F_{ENO} 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

alérgicos) y 39 voluntarios sanos. Todos los pacientes se sometieron a medición de F_{ENO}, pruebas cutáneas con alérgenos inhalantes comunes, análisis de proteína catiónica sérica del eosinófilo (ECP), eosinofilia en sangre y espirometría flujo-volumen. Cuando el volumen espiratorio forzado en el primer segundo (FEV_1) fue inferior a un 80% de lo previsto, se valoró la reversibilidad de la obstrucción de las vías respiratorias con un agonista ß$_2$. A los pacientes asmáticos con un valor de referencia de FEV_1 de más de un 70% de lo previsto, se les realizó una prueba de provocación bronquial no específica con histamina.
Resultados: Comparado con los voluntarios sanos, el F_{ENO} estaba elevado en ambos grupos de asmáticos. El F_{ENO} del grupo de asmáticos alérgicos estaba más elevado que el de los asmáticos sin alergias. En ambos grupos de asmáticos, el F_{ENO} se correlacionaba de forma significativa con la hiperreactividad bronquial a la histamina, reversibilidad de la obstrucción de las vías respiratorias, niveles séricos de ECP y eosinofilia sanguínea. El F_{ENO} no se relacionó con los valores de referencia FEV_1 en ninguno de los 2 grupos de asmáticos. En un 31% de los pacientes no alérgicos y en un 9% de los alérgicos, el F_{ENO} fue inferior a 20 partes por billón.
Conclusiones: Sugerimos que la medición del F_{ENO} podría ser clínicamente útil para los asmáticos sin un tratamiento previo con esteroides y que debería usarse más en la práctica clínica. La medición del F_{ENO} es un procedimiento no invasivo, simple y reproducible, cuyos resultados se correlacionan con otros métodos comúnmente usados para el diagnóstico del asma. No obstante, es importante destacar que algunos pacientes, especialmente aquéllos con asma no alérgica, no presentan niveles elevados de $F_{ENO.}$

Palabras clave: Asma. Óxido nítrico exhalado. Pruebas de función pulmonar. Proteína catiónica del eosinófilo. Eosinofilia sanguínea. IgE.

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 (F_{ENO}) 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 (F_{ENO}) 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 F_{ENO} 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 F_{ENO} 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 F_{ENO} 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 F_{ENO}—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 ß$_2$-agonists. Asthmatic patients who had been treated with inhaled steroids in the past were excluded from the study. F_{ENO} 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 (FEV_1) was less than 80% of predicted, reversibility with an inhaled ß$_2$-agonist (400 µg of salbutamol) was assessed. A histamine challenge was carried out in patients with baseline FEV_1 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 $F_{ENO,}$ 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 F_{ENO} 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

F_{ENO} 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 F_{ENO} 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 F_{ENO}.

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 $ß_2$-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 µg 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 ± 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.

Serum ECP and peripheral blood eosinophilia were

Table 1. Characteristics of Study Subjects and Healthy Volunteers*

Characteristics	Allergic Asthma	Nonallergic Asthma	*P* (Allergic vs Nonallergic Asthma)	Healthy Volunteers
Number of patients	56	45	–	39
Asthma severity, mild/moderate, ratio	42/14	29/16	–	–
Sex, F/M, ratio	31/25	28/17	–	24/15
Age, y	32±12	40±12	>.05	33.5±15.2
Duration of symptoms, y	6.2±4.3	4.9±4.2	>.05	–
Baseline FEV_1, % predicted	87.8±16.7	82.6±12.2	.09	101±4.8†,‡
ΔFEV_1, %	27.2±11.6	20.2±7.1	.02	–
PC20histamine FEV_1, mg/mL	2.0±1.93	2.8±1.96	.07	–
Blood eosinophil count, cells/mm^3	246.6±105.3	211±97.8	.06	119.2±36.5†,‡
Serum ECP, µg/L	15.16±8.61	11.84±5.72	.09	4.38±4.7†,‡
Total Serum IgE, kU/L	212±145	78±69	.004	65±51†
BPT	49	37	–	–
Reversibility test	19	20	–	–

* Data are shown as means ± SD, unless otherwise indicated. FEV_1 indicates forced expiratory volume in the first second; ΔFEV_1, increase in FEV_1 after inhaling 400 µg salbutamol; PC20histamine 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, $P < .05$

Table 2. Correlations Between Serum ECP and F_{ENO} or Results of Pulmonary Function Tests*

Study Groups	F_{ENO}		FEV_1		PC20 Histamine FEV_1		ΔFEV_1	
Allergic asthma	***r* = 0.57**	***P* = .0005**	*r* = –0.11	*P* = .39	***r* = –0.37**	***P* = .007**	*r* = 0.14	*P* = .56
Nonallergic asthma	***r* = 0.47**	***P* = .001**	*r* = –0.1	*P* = .49	***r* = –0.45**	***P* = .005**	*r* = 0.19	*P* = .15
Healthy controls	*r* = –0.06	*P* = .7	*r* = –0.02	*P* = .89				

*F_{ENO} indicates fractional concentration of exhaled nitric oxide; ECP, eosinophil cationic protein; FEV_1, forced expiratory volume in the first second; ΔFEV_1, increase in FEV_1 after inhaling 400 µg salbutamol; PC20histamine FEV_1, provocative concentration of histamine that caused a 20% reduction in FEV_1.

Table 3. Correlations Between Blood Eosinophil Count and F_{ENO} or Results of Pulmonary Function Tests*

Study Groups	F_{ENO}		FEV_1		PC20 Histamine FEV_1		ΔFEV_1	
Allergic asthma	***r* = 0.69**	***P* = .0002**	*r* = –0.03	*P* = .82	***r* = –0.41**	***P* = .003**	*r* = 0.26	*P* = .27
Nonallergic asthma	***r* = 0.64**	***P* = .0001**	*r* = –0.15	*P* = .15	***r* = –0.42**	***P* = .008**	*r* = 0.36	*P* = .09
Healthy controls	*r* = –0.05	*P* = .72	*r* = –0.16	*P* = .3				

*F_{ENO} indicates fractional concentration of exhaled nitric oxide; FEV_1, forced expiratory volume in the first second; ΔFEV_1, increase in FEV_1 after inhaling 400 µg salbutamol; PC20histamine FEV_1, provocative concentration of histamine that caused a 20% reduction in FEV_1.

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 F_{ENO} levels and serum ECP (Table 2) and between F_{ENO} 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 FEV_1 ($PC20FEV_1$) and both serum ECP (Table 2) and blood eosinophil count (Table 3).

The F_{ENO} 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 F_{ENO} 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 F_{ENO} 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, F_{ENO}

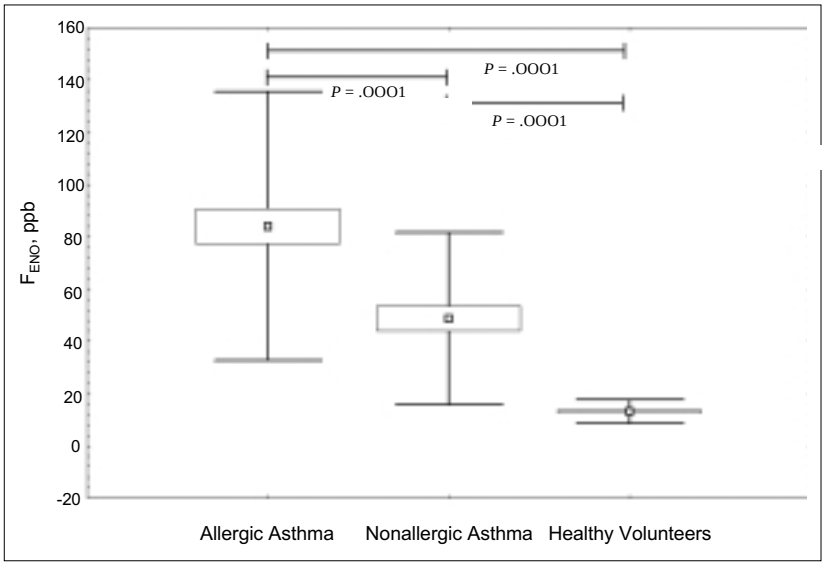

Figure 1. Exhaled NO levels in the groups of asthma patients and healthy volunteers. F_{ENO} indicates fractional concentration of exhaled nitric oxide; ppb, parts per billion.

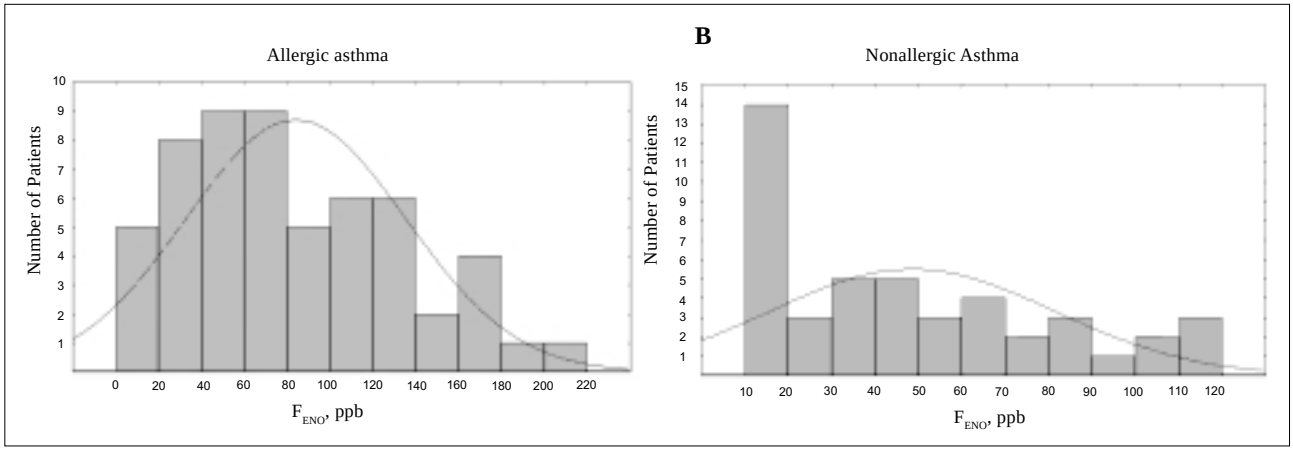

Figure 2. Histograms showing F_{ENO} in allergic asthma (*A*) and nonallergic asthma (*B*) patients. F_{ENO} indicates fractional concentration of exhaled nitric oxide; ppb, parts per billion.

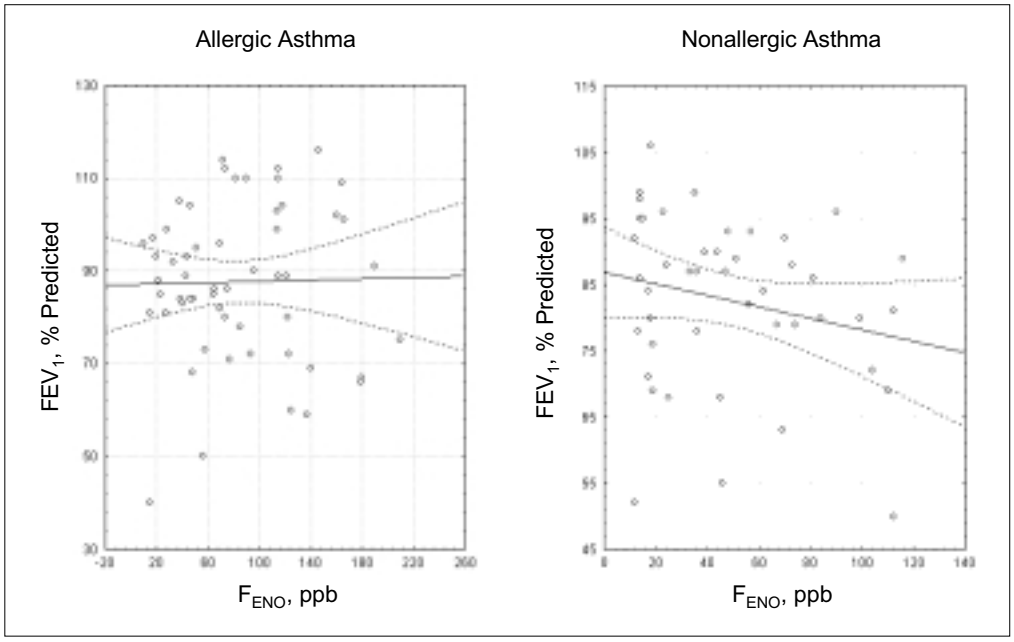

Figure 3. Relationship between F_{ENO} and baseline FEV_1 value. No significant correlation was observed for either allergic asthma (r = 0.02089, P = .87) or nonallergic asthma (r = –0.2244, P = .13). F_{ENO} indicates fractional concentration of exhaled nitric oxide; ppb, parts per billion; FEV_1, forced expiratory volume in the first second.

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).

F_{ENO} 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 F_{ENO} and the time-course of disease symptoms or total serum IgE in any of the groups studied. Also, there was no correlation between F_{ENO} and baseline FEV_1 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, F_{ENO} displayed a significant negative correlation with the $PC20FEV_1$ for histamine (allergic asthma, r = – 0.62, P = .00002; nonallergic asthma, r = –0.41, P = .01; Figure 4).

F_{ENO} levels displayed a significant correlation with the reversibility of airway obstruction after ß$_2$-agonist inhalation in both groups of patients (allergic asthma, r = 0.51, P = .02; nonallergic asthma, r = 0.47, P = .03; Figure 5).

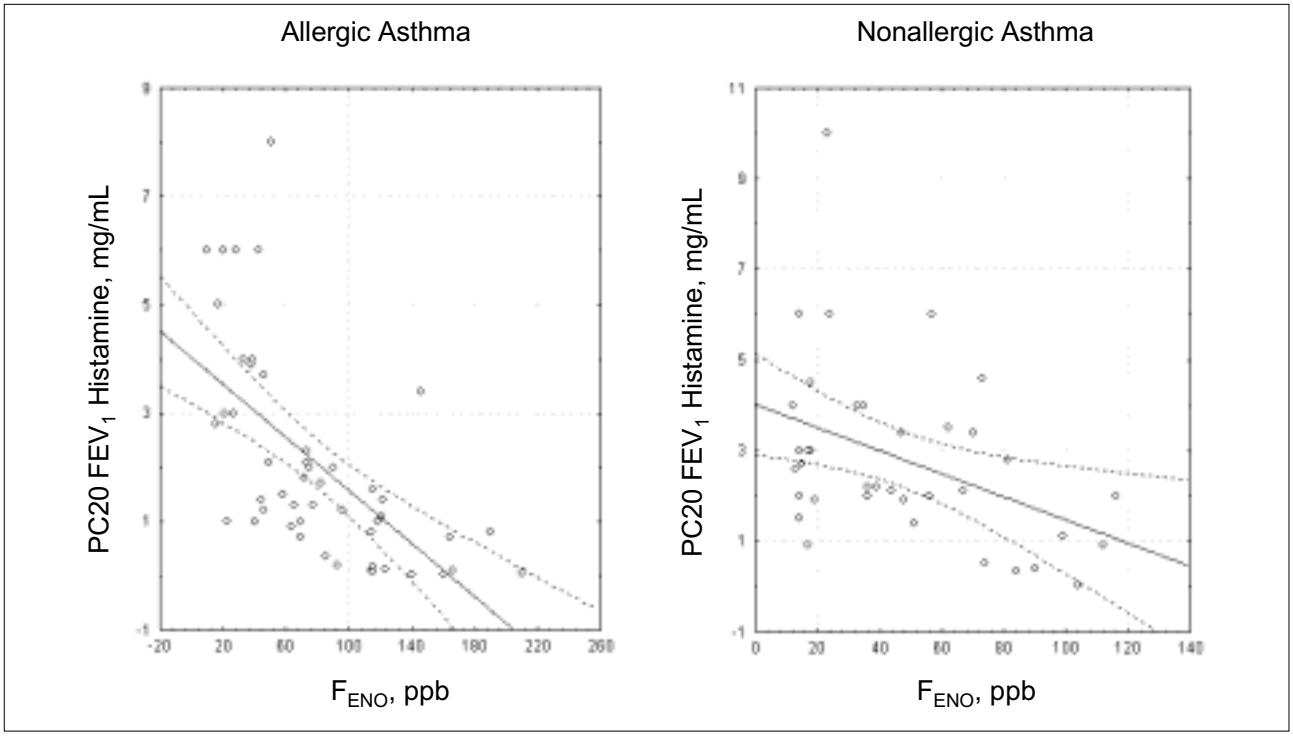

Figure 4. Relationship between F_{ENO} and PC20histamine FEV_1 in both groups of asthma patients. Significant correlations were observed for both allergic asthma ($r = -0.6218$, $P = .00002$) and nonallergic asthma ($r = -0.4101$, $P = .01$). F_{ENO} indicates fractional concentration of exhaled nitric oxide; ppb, parts per billion; PC20histamine FEV_1, provocative concentration of histamine that caused a 20% reduction in FEV_1.

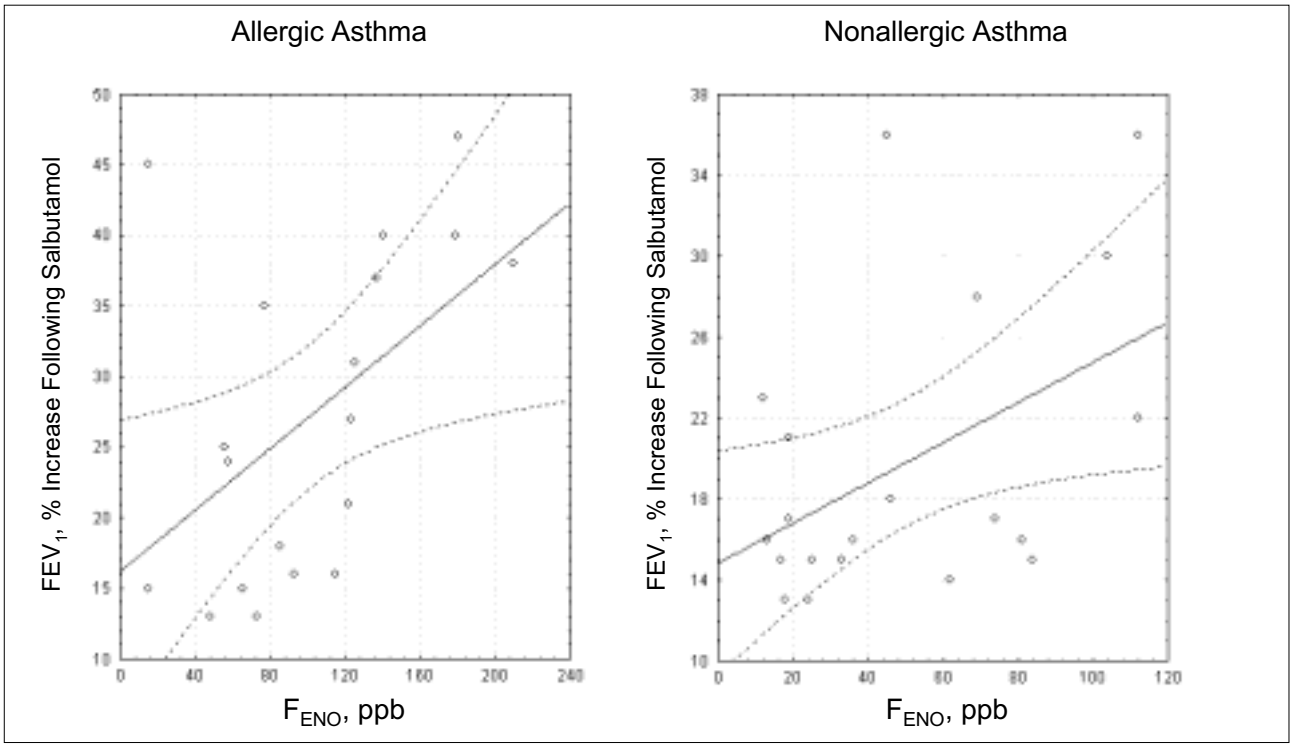

Figure 5. Correlations between F_{ENO} and reversibility of airway obstruction following administration of 400 μg of salbutamol. Significant correlations were observed for both allergic asthma ($r = 0.51061$, $P = .02$) and nonallergic asthma ($r = 0.47241$, $P = .03$). F_{ENO} indicates fractional concentration of exhaled nitric oxide; ppb, parts per billion; FEV_1, forced expiratory volume in the first second.

Discussion

In patients with asthma, elevated F_{ENO} 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 F_{ENO} 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 F_{ENO} correlates well with conventional markers of airway inflammation. F_{ENO} 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 F_{ENO} 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 F_{ENO} 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 FEV_1 or the reversibility of airway obstruction.

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

Our results showed significantly higher F_{ENO} 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 F_{ENO} than individuals with asthma alone.

The results of F_{ENO} measurements allow assessment of the clinical usefulness of this test and suggest that it is more useful in allergic asthmatics. Predefined normal values for F_{ENO} (< 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 F_{ENO} 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 hyperresponsiveness 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 F_{ENO} 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 F_{ENO} 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 F_{ENO} 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. FEV_1 is the most commonly used parameter in diagnosis and treatment monitoring in asthmatic patients. Some authors are doubtful about the usefulness of FEV_1 assessment, especially in subjects with mild asthma [11]. In general, F_{ENO} does not correlate with lung function parameters in stable asthma patients. F_{ENO} 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 F_{ENO} and FEV_1. 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 F_{ENO} and reversibility of airway obstruction.

Conclusions

Assessment of F_{ENO} 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 F_{ENO} values. F_{ENO} correlates with the results of other tests used in the diagnosis of asthma (assessment of bronchial hyperreactivity and reversibility of airway obstruction). F_{ENO} is not correlated with baseline FEV_1; this observation is very important in patients with mild asthma, since spirometric indices are not very useful in some such patients. Measurement of F_{ENO} is a rapid, simple, reproducible test, and it can be performed in all patients in whom spirometry can be carried out. High values for F_{ENO} 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.

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