ORIGINAL ARTICLES

Contribution of Exhaled Nitric Oxide Measurements to Abbreviated Bronchial Challenge Test Protocols

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OBJECTIVE: The bronchial challenge test is commonly used to diagnose asthma but it is a tedious, time-consuming procedure. Although in recent years, several shortened methods have been proposed, it has been shown that they can give rise to exaggerated bronchoconstriction. The aims of the present study were *a)* to determine the frequency of exaggerated bronchoconstriction in patients with asthma following the application of a shortened bronchial challenge test, and *b)* to determine if the fraction of exhaled nitric oxide (FE_{NO}) can be used to predict the onset of exaggerated bronchoconstriction.

PATIENTS AND METHODS: We performed a prospective study of 210 patients with asthma in whom FE_{NO} levels were measured in accordance with the abbreviated protocol recommended by the European Respiratory Society (ERS). Exaggerated bronchoconstriction was defined as a decrease of more than 20% in forced expiratory volume in 1 second after the first challenge, after a skipped dose, or after administration of saline. A receiver operating characteristic (ROC) curve was generated to determine the best FE_{NO} cutoff value for predicting exaggerated bronchoconstriction. The pretest probability of developing exaggerated bronchoconstriction was also calculated using Bayes' theorem.

RESULTS: The frequency of exaggerated bronchoconstriction in our series was 30%. Patients who developed exaggerated bronchoconstriction had significantly higher FE_{NO} levels than those who did not (32.6 vs 16.2 parts per billion [ppb]). The chosen FE_{NO} cutoff of 19.5 ppb had a sensitivity of 80%, a specificity of 77%, and a negative predictive value of 88%. The area under the ROC curve was 0.83 (95% confidence interval, 0.77-0.89).

CONCLUSIONS: The abbreviated bronchial challenge test recommended by the ERS led to exaggerated bronchoconstriction in 30% of the patients studied. FE_{NO} measurements could possibly be used to identify patients at increased risk of exaggerated bronchoconstriction. The shortened challenge test can be performed safely in individuals with a FE_{NO} of <19.5 ppb.

Key words: *Exhaled nitric oxide. Bronchial challenge test. Receiver operating characteristic (ROC) curves. Exaggerated bronchoconstriction.*

Aportaciones del óxido nítrico exhalado a los procedimientos abreviados de las pruebas de provocación bronquial

OBJETIVO: La prueba de provocación bronquial es un procedimiento habitual en el diagnóstico del asma, pero su realización resulta larga y tediosa. Por ello se han propuesto métodos que acortan su duración. Sin embargo, en los últimos años se ha señalado que dichos métodos pueden dar lugar a broncoconstricciones excesivas (BE). Los objetivos del presente estudio han sido: a) determinar la frecuencia de BE en pacientes con asma tras la aplicación del método abreviado de la prueba de provocación bronquial, y b) cuantificar si la determinación de óxido nítrico en aire exhalado (ONE) puede predecir la aparición de BE.

PACIENTES Y MÉTODOS: Se ha realizado un estudio prospectivo sobre 210 asmáticos a quienes se determinó el ONE y se realizó una prueba de provocación bronquial siguiendo el protocolo abreviado de la European Respiratory Society (ERS). Se definió BE como una caída superior al 20% del volumen espiratorio forzado en el primer segundo tras la primera dosis, después de suprimir una dosis o tras el diluyente. Se construyó una curva de eficacia diagnóstica para determinar el mejor punto de corte del ONE para predecir BE y se calculó la probabilidad preprueba de presentar BE, siguiendo el teorema de Bayes.

RESULTADOS: La frecuencia de BE en nuestra serie fue del 30%. Hubo diferencias significativas en el ONE, siendo la concentración más elevada en el grupo que presentó BE (32,6 frente a 16,2 ppb). El punto de corte de ONE elegido fue 19,5 ppb, con una sensibilidad del 80%, especificidad del 77% y valor predictivo negativo del 88%. El área bajo la curva de eficacia diagnóstica fue de 0,83 (intervalo de confianza del 95%, 0,77-0,89).

CONCLUSIONES: La prueba de provocación bronquial abreviada que recomienda la ERS da lugar a un 30% de BE. La determinación de ONE podría identificar a los pacientes con mayor probabilidad de presentar BE. Una concentración de ONE inferior a 19,5 ppb permite realizar la prueba de provocación bronquial abreviada con seguridad.

Palabras clave: *Óxido nítrico exhalado. Prueba de provocación bronquial. Curvas de eficacia diagnóstica. Broncoconstricción excesiva.*

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Introduction

Although the histamine/methacholine bronchial challenge test was first standardized in the 1970s,[1,2] alternative protocols have since been proposed to shorten what is considered to be an excessively long and tedious test. Thanks to these modifications, the test can now be started with higher doses of bronchoconstrictor than those recommended in the original protocol; for each patient, the starting concentration is determined following consideration of baseline forced expiratory volume in 1 second (FEV_1), current asthma medication, and the presence or not of respiratory symptoms.[3,4] Although the original test has been shown to be quite safe in practice,[5,6] several studies have indicated that the shortened version may occasionally lead to exaggerated bronchoconstriction, with sharp falls in FEV_1.[7-10] While attempts have been made to identify the determinants of exaggerated bronchoconstriction, none of the variables considered to date (such as lung function, a history of atopy or rhinitis, age, and previous asthma symptoms) have proven to be unequivocally correlated.[8,9] To the best of our knowledge, the importance of the extent of bronchial inflammation at the time of the challenge test has never been studied. We believe that this should be taken into account as bronchial inflammation plays a role in the pathogenesis of airway hyperresponsiveness.[11]

The present study had 2 aims: *a)* to determine the frequency of exaggerated bronchoconstriction in people with asthma following the application of the shortened version of the histamine challenge test proposed by the European Respiratory Society[3] (ERS), and *b)* to quantify the extent to which the fraction of exhaled nitric oxide (FE_{NO}), a marker for airway inflammation,[12] is capable of predicting the onset of exaggerated bronchoconstriction.

Patients and Methods

Study Design

We performed a prospective study of 210 patients (133 women and 77 men) with a mean (SD) age of 33.3 (12.5) years who consecutively visited the lung function laboratory at Hospital Universitario La Fe in Valencia, Spain with clinically suspected or confirmed asthma of varying degrees of severity. Once the patients had been informed of the purpose of the study and signed the corresponding consent form, they were interviewed by one of the authors about their symptoms, the time since onset of these, the treatment of their asthma, and their smoking history. FE_{NO} levels were then measured and a bronchial challenge test administered.

Measurement of FE_{NO} Concentrations

FE_{NO} concentrations were measured online in accordance with international recommendations[13] using a chemiluminescence analyzer with a sensor for $PaCO_2$ in exhaled air (LR 2000; Logan Research, Rochester, UK). The patient, in a sitting position and not wearing a nose clip, exhaled from a full lung at a constant flow of 50 mL/s through a mouthpiece with a resistance of 20 cm H_2O for 10 seconds. End-expiratory plateau concentrations of FE_{NO} were measured. Three valid measurements were obtained for each patient; a maneuver was considered valid if it did not differ from the preceding maneuver by more than 2.5 parts per billion (ppb) or 10%. The final measurement was obtained by calculating the average of the 3 maneuvers.

Bronchial Challenge Test

The bronchial challenge test was performed with histamine using the 2-minute tidal breathing dosing protocol.[3] Once baseline spirometry had been performed, the patient inhaled saline (0.9% saline solution) for 2 minutes and was then administered a double dose of histamine until FEV_1 decreased by 20% or more with respect to baseline, or until a dose of 16 mg/mL of histamine was reached.

The following criteria were used to determine the initial histamine dose (Table 1)[3]: *a)* in patients with a baseline FEV_1 greater than 70%, FEV_1/forced vital capacity (FVC) greater than 80%, and a decrease in FEV_1 of less than 10% after the administration of saline, the starting concentration was 2 mg/mL for those not taking any medication, 1 mg/mL for those using bronchodilators occasionally, 0.25 mg/mL for those using bronchodilators daily, and 0.125 mg/mL for those receiving inhaled or oral corticosteroids; *b)* in patients with a baseline FEV_1 of less than 70%, FEV_1/FVC of less than 80%, and a decrease in FEV_1 of less than 10% after the administration of saline, the starting concentration was 0.03 mg/mL for those receiving inhaled or oral corticosteroids and 0.125 mg/mL for those not receiving corticosteroids; and *c)* in patients with a decrease in FEV_1 of more than 10% after the administration of saline, the starting concentration was 0.03 mg/mL. If the decrease in FEV_1 following a challenge was less than 5%, the next dose was skipped, except in the case of 2 mg/mL doses. We decided not to skip a concentration step at that point to maximize patient safety as the 4-fold increase in concentration between 2 successive doses would have been excessive in our opinion. On completion of the test, the patients received 600 µg of salbutamol through a pressurized metered-dose inhaler with a spacer device.

Exaggerated bronchoconstriction was defined as a decrease in FEV_1 of 20% or more with respect to baseline after the first challenge, after a skipped dose, or after the administration of saline. The concentration of histamine capable of causing a 20% decrease in FEV_1 (PC_{20}) was calculated using semilogarithmic interpolation.

TABLE 1
Criteria Used to Determine the Initial Histamine Dose for the Shortened Version of the Bronchial Challenge Test as Recommended by the European Respiratory Society[3]

Baseline Spirometry	Starting Histamine Dose (mg/mL)
FEV_1 >70%, FEV_1/FVC >80%, no response to saline	
No medication	2
Bronchodilators occasionally	1
Bronchodilators daily	0.25
Inhaled corticosteroids	0.125
FEV_1 <70%, and/or FEV_1/FVC <80%, no response to saline	
Inhaled corticosteroids	0.03
Other patients	0.125
Decrease in FEV_1 of <10% after saline	0.03

Abbreviations: FEV_1 indicates forced expiratory volume in 1 second; FVC, forced vital capacity.

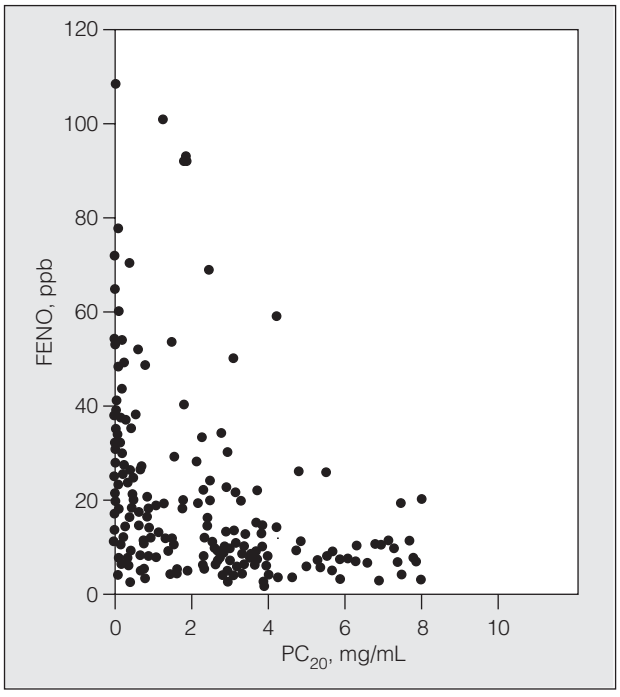


Figure 1. Correlation between the concentration of histamine causing a 20% decrease in forced expiratory volume in 1 second (PC_{20}) and the fraction of nitric oxide in exhaled air (FE_{NO}) values for the entire study population (r=–0.54, *P*<.0001). ppb indicates parts per billion.

Statistical Analysis

Continuous variables were described using mean (SD) values and categorical variables using absolute frequencies and percentages. Means were compared using the *t* test for independent groups following confirmation of normal distribution. Frequencies were compared using the χ^2 test and correlations analyzed using the Pearson correlation coefficient (*r*). Statistical significance was set at a value of *P*<.05.

In order to determine the concentration of FE_{NO} capable of predicting exaggerated bronchoconstriction, we calculated sensitivity, specificity, positive predictive power, and negative predictive power, with their 95% confidence intervals (CI), for each FE_{NO} measurement. With the resulting data, we generated a receiver operating characteristic (ROC) curve to find the best FE_{NO} cutoff (value with the greatest sensitivity and specificity) and to calculate the area under the curve (AUC), with its 95% CI, to estimate overall diagnostic accuracy. AUCs with a value of close to 1 indicated excellent ability to discriminate.[14] Using the resulting cutoff, we determined the posttest probability of developing bronchoconstriction during the bronchial challenge test for patients with FE_{NO} concentrations higher than the cutoff (positive predictive value) and lower than the cutoff (1–negative predictive value). Using Bayes' theorem, we also calculated the posttest probability of not developing bronchoconstriction following a negative predictive value for each possible pretest probability.[14]

Positive predictive value = (pretest probability × sensitivity)/(pretest probability × sensitivity) + [(1-pretest probability) × (1–specificity)]

Negative predictive power = (1–pretest probability × specificity/[(1–pretest probability × specificity] + [pretest probability × (1–sensitivity)]

Pretest probability refers to estimates made by clinicians and expressed in terms of the likelihood of a test result before the test is performed. In the present study, it referred to the probability that a patient would develop bronchoconstriction during the challenge test in the opinion of the clinician on the basis of his/her experience and the data available and before the test was actually performed. With the above formulas, it is feasible to calculate the positive and negative predictive values for each possible pretest probability (from 0.1 to 1) using the sensitivity and specificity determined for the best FE_{NO} cutoff.

Tests were performed using version 12.0 of the statistical software package SPPS (Chicago, Illinois, USA), except for pretest and posttest probabilities, which were calculated manually with a Microsoft Excel spreadsheet used to perform mathematical operations and plot the ROC curve.

Results

The PC_{20} was less than 8 mg/mL for all patients, meaning that asthma was confirmed in all the study patients who visited the lung function laboratory with clinically suspected asthma. In accordance with the Spanish guidelines for the management of asthma (GEMA),[15] at the time of the study, 79 patients had intermittent asthma, 81, mild persistent asthma, 45, moderate persistent asthma, and 5, severe persistent asthma.

The most common starting concentrations of histamine were 2 mg/mL (83 patients), 0.125 mg/mL (n=71), and 0.5 mg/mL (n=33). The starting concentration was 1 mg/mL for 12 patients, 0.25 mg/mL for 7 patients, and 0.03 mg/mL for 4 patients. In 38.5% of patients (n=71), a concentration step was skipped during the challenge test in accordance with the protocol.

None of the patients developed exaggerated bronchoconstriction after administration of saline but 64 patients (30.5%) did after a histamine challenge (52 after the first challenge and 12 after a skipped dose). Decreases in FEV_1 were greater than 30% in 30 patients (14.2%) and greater than 40% in 14 patients (6.6%): 7 of these experienced a decrease of greater than 60%.

Exaggerated bronchoconstriction was less frequent in those receiving inhaled corticosteroids than in those not receiving these drugs (20% of patients vs 39%, respectively; *P*<.01). The FE_{NO} value was significantly higher in patients with exaggerated bronchoconstriction than in those without (32.7 [23.5] ppb vs 16.2 [21.5] ppb, respectively; *P*<.0001). PC_{20} values were also different between the 2 groups (0.45 [0.66] mg/mL vs 3.09 [2.19] mg/mL, respectively; *P*<.0001) (Table 2). FE_{NO} and PC_{20} were negatively correlated for the group as a whole (*r*=–0.54, *P*<.0001) (Figure 1). The only other significant differences detected between patients with exaggerated bronchoconstriction and those without were related to FEV_1/FVC, asthma severity, and treatment with inhaled corticosteroids.

Figure 2 shows the ROC curve for predicting exaggerated bronchoconstriction using the FE_{NO} values obtained (AUC=.083; 95% CI, 0.77-0.89). The best cutoff was obtained for a FE_{NO} of 19.5 ppb, with a sensitivity of 0.8 (95% CI, 0.7-0.9), a specificity of 0.77 (95% CI, 0.7-0.84), a positive predictive power of 0.58, and a negative predictive power of 0.88. Finally, Figure 3 shows how using this cutoff changed the posttest likelihood of exaggerated

bronchoconstriction following a positive result (FE_{NO} ≥19.5 ppb) and a negative result (FE_{NO} <19.5 ppb). In our case, the best result was obtained for a pretest probability of approximately 51% (95% CI, 22%-79%).

Discussion

The findings of the present study show that *a)* the abbreviated protocol of the bronchial challenge test recommended by the ERS[3] causes exaggerated bronchoconstriction in an appreciable proportion of patients with asthma; and *b)* such episodes could be prevented, with reasonable confidence by measuring FE_{NO} concentrations prior to the test.

The safety of shortened versions of the bronchial challenge test has been evaluated previously, and even though there is agreement that the risk involved is very low, all the publications have noted that some patients experience sharp drops in FEV_1.[7-9,16] In our series, around 30% of patients developed exaggerated bronchoconstriction. This is higher than rates reported in the literature (<10%). We believe that this discrepancy is due to 2 factors:

1. Most of the studies published to date have involved both patients with asthma and members of the general public. Our series included the former but not the latter; some of the patients had confirmed asthma under treatment at the time of the study, and others had suspected asthma that was confirmed later.

TABLE 2
Characteristics of Patients (n=210) Grouped According to Whether or Not They Developed Exaggerated Bronchoconstriction[a]

Clinical Features	Exaggerated Bronchoconstriction	
	No	Yes
Patients	146 (69.52)	64 (30.48)
Age, y	35.46 (13.3)	31.24 (11.2)
Men	61 (41.5)	16 (25.4)
Smoking		
Nonsmokers	76 (52.1)	37 (57.8)
Ex-smokers	36 (24.7)	10 (15.6)
Smokers	34 (23.3)	17 (26.6)
Baseline FEV_1, % of predicted	101.26 (12.9)	98.07 (11.14)
FEV_1/FVC, % of predicted[b]	97.5 (7.2)	98.07 (10.3)
FE_{NO}, ppb	16.23 (21.53)	32.69 (23.5)
PC_{20}, mg/mL	2.95 (2.18)	0.32 (0.55)
Asthma severity[b,c]		
Intermittent	44 (30.1)	35 (54.7)
Mild	65 (44.5)	18 (28.1)
Moderate	33 (22.6)	9 (14.1)
Severe	4 (2.7)	2 (3.1)
Time since diagnosis of asthma, y	4.9 (7)	5.7 (7)
Treatment with inhaled corticosteroids[b]		
Yes	84 (57.5)	22 (34.4)
No	62 (42.5)	42 (65.6)

Abbreviations: FE_{NO} indicates nitric oxide in exhaled air; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; ppb, parts per billion; PC_{20}, concentration of histamine causing a 20% decrease in FEV_1.
[a]Data are given as mean (SD) or as number and percentage of patients.
[b]Significant intergroup difference (*P*<.01).
[c]Determined according to the Spanish guidelines for the management of asthma (GEMA).

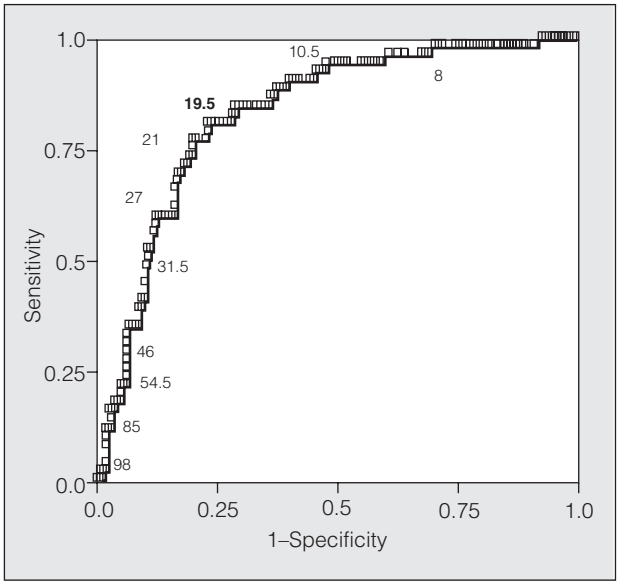
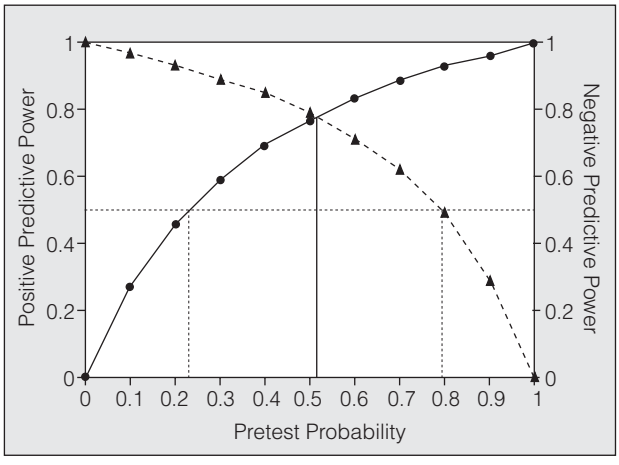


Figure 2. Receiver operating characteristic curve for fraction of exhaled nitric oxide to predict exaggerated bronchoconstriction. The cutoff is the point with maximum sensitivity (0.8) and specificity (0.77). Some of the intermediate data points are also shown in the figure.

2. The definition of exaggerated bronchoconstriction varies from one study to the next. Kremer et al,[7] for example, defined it as a decrease in FEV_1 of 40% or more. They adapted the starting concentration of histamine in accordance with the patient's symptoms and stopped the test when FEV_1 decreased by at least 18% in order to prevent the next dose from causing excessive reduction in lung function. They reported bronchoconstriction in 3.1%, a rate which is very similar to the rate of 6.6% in our study for a FEV_1 decrease of 40% or more. Troyanov et al[8] defined exaggerated bronchoconstriction as a decrease of more than 20% in FEV_1 after the administration of saline



Posttest probability of a patient experiencing exaggerated bronchoconstriction after a positive result (continuous line with circles) or of not after a negative result (dotted line with triangles), using a fraction of nitric oxide in exhaled air of 19.5 parts per billion as the cutoff. The point at which both lines intersect corresponds to the pretest probability for the usefulness of this cutoff.

or of more than 30% after the first challenge with methacholine. Their rate of exaggerated bronchoconstriction based on these criteria was quite similar to ours for a decrease in FEV_1 of greater than 30% (10% vs 14%, respectively). The 2 cohorts were similar in that almost half of the patients studied were under treatment with inhaled corticosteroids. A careful analysis of the data published by Troyanov[8] shows that their exaggerated bronchoconstriction rate would have risen to 21% if they had only considered patients with a PC_{20} of greater than 32 mg/mL. Finally, in a retrospective study involving 1000 bronchial challenge tests that used the same criteria as we did, Cockcroft et al[16] detected an exaggerated bronchoconstriction rate of 3%, which rose to 5.7% for patients with a PC_{20} of less than 8 mg/mL. The most common initial dose in their study was 1 mg/mL, compared to 2 mg/mL in ours. We do not know if their subgroup of patients with a PC_{20} of less than 8 mg/mL (the population most similar to the patients we studied) had the same starting concentration as we used. If that were the case, it would point to an explanation of why Cockcroft et al observed a lower rate of exaggerated bronchoconstriction than we did.

In view of these considerations, we believe that exaggerated bronchoconstriction occurs with greater frequency during the shortened version of the bronchial challenge test than is believed, and that our results are a closer reflection of what actually occurs during lung function testing. It would therefore be useful to have a tool capable of detecting whether patients with confirmed or suspected asthma were at risk of developing exaggerated bronchoconstriction during a shortened challenge test. Our findings indicate that the FE_{NO} test could do just this. It is a simple, non-invasive, reproducible, and widely standardized test that indicates the extent of eosinophilic inflammation in the respiratory tree and, moreover, is related to other markers of eosinophilic inflammation and bronchial hyperresponsiveness.[12,13,17,18] FE_{NO} measurements have been proposed for use in patients with asthma to diagnose disease, predict exacerbations, and check the efficacy of and the need for antiinflammatory treatment.[19] Our findings show that this test might have an even wider field of application. According to our data, FE_{NO} levels of less than 19.5 ppb indicate a low risk of developing exaggerated bronchoconstriction when an abbreviated bronchial challenge protocol is used.

We generated a ROC curve to establish this best cutoff of 19.5 ppb, which had high sensitivity (0.8) and specificity (0.7). The use of higher FE_{NO} figures would have increased sensitivity but at the expense of losing specificity, and this would have reduced the chance of identifying patients at risk of developing exaggerated bronchoconstriction during the shortened test. We also calculated the posttest probability of developing exaggerated bronchoconstriction following a positive result (FE_{NO} >19.5 ppb) and a negative result (FE_{NO} <19.5 ppb), finding that the best result was obtained for situations in which there was a pretest probability of 51.2%. In other words, measuring FE_{NO} concentrations prior to a shortened bronchial challenge test would be most useful when a clinician estimated a 50% chance of a patient developing exaggerated bronchoconstriction.

The results confirm our initial hypothesis that, because eosinophilic airway inflammation is involved in the pathogenesis of bronchial hyperresponsiveness, exaggerated bronchoconstriction could be predicted using an inflammation marker. In our series, we observed a significant negative correlation between FE_{NO} and PC_{20} values, and we also found that fewer patients receiving corticosteroids developed exaggerated bronchoconstriction than those not receiving these drugs. One question worth considering is whether or not this association is powerful enough to warrant replacing the bronchial challenge test with simple FE_{NO} measurements. We think that it is not, primarily for 2 reasons: *a)* bronchial hyperresponsiveness is more than just inflammation,[20] and *b)* not all inflammation in asthma is eosinophilic. We believe that patient safety can be increased, however, by measuring FE_{NO} concentrations prior to performing a shortened version of the bronchial challenge test. We did not analyze how many patients had eosinophilic asthma in our series, but it would be interesting to do so in a similar study in the future. As far as the presumed protective role played by inhaled corticosteroids is concerned, it should be remembered that the test protocol states that patients receiving corticosteroids should be given a lower starting concentration than those not receiving these drugs. To our understanding, it logically follows that the risk of experiencing a sharp drop in FEV_1 is much lower in patients who have received a lower concentration of histamine.

In conclusion, in our series the abbreviated version of the bronchial challenge test recommended by the ERS[3] caused exaggerated bronchoconstriction in a third of patients with asthma. FE_{NO} measurements taken prior to this test could identify, with reasonable confidence, patients at risk of such episodes. According to our findings, a FE_{NO} value of less than 19.5 ppb indicates that the test can be performed safely; higher values, in contrast, indicate that the test should be started using low concentrations of bronchoconstrictor agent to prevent the onset of exaggerated bronchoconstriction.

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