

Cutoff Point for Exhaled Nitric Oxide Corresponding to 3% Sputum Eosinophils

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■ Abstract

Background: The eosinophilic asthma phenotype (sputum eosinophils $\geq 3\%$) indicates a good response to corticosteroids and T_H2 immunomodulators. Exhaled nitric oxide (FeNO) is rapidly measured by portable devices, and although it is not a selective marker of eosinophilic inflammation, several studies have demonstrated a strong correlation with it. We investigated which FeNO value was the best fit with 3% sputum eosinophils in asthma patients.

Methods: We included 129 consecutive, nonsmoking asthmatics who underwent skin tests, FeNO quantification (NIOX MINO), spirometry, and induced sputum analysis and completed the Asthma Control Test questionnaire. Receiver operating characteristic curves were constructed, and logistic regression analysis was performed.

Results: Symptoms were detected more frequently in the eosinophilic asthma group, as were higher airway obstruction and sensitivity to hypertonic saline. The FeNO cutoff point of 21 ppb was the best fit with 3% sputum eosinophilia. This value behaved better among corticosteroid-naïve patients (sensitivity, 97%; specificity, 58%; positive predictive value, 86%; negative predictive value, 88%) than among those receiving corticosteroids (sensitivity, 81%; specificity, 25%; positive predictive value, 74%; negative predictive value, 33%).

Conclusion: FeNO ≥ 21 ppb is associated with airway eosinophilia. In corticosteroid-naïve patients, FeNO < 21 ppb enables us to rule out airway eosinophilia.

Key words: Asthma. Nitric oxide cut point. Eosinophilic phenotype. ROC curves. Sputum eosinophils.

■ Resumen

Antecedentes: El fenotipo de asma eosinofílica (eosinófilos en esputo $\geq 3\%$) es un marcador de buena respuesta a corticosteroides y fármacos T_H2 inmunomoduladores. La Fracción Exhalada de Óxido Nítrico (FE_{NO}) se puede medir de forma rápida y sencilla con dispositivos portátiles, y si bien no es un marcador selectivo de inflamación eosinofílica, numerosos estudios han mostrado que guarda una buena relación con ella. En el presente estudio, hemos evaluado qué valor de FENO discrimina mejor la eosinofilia en esputo $\geq 3\%$.

Métodos: Incluimos 129 asmáticos no fumadores consecutivos a quienes se realizaron pruebas cutáneas, medición de FENO (Niox Mino), espirometría forzada e inducción de esputo. Los pacientes autocompletaron el Test de Control de Asma (ACT). Se realizaron curvas ROC y estudio estadístico de regresión logística.

Resultados: El grupo con asma eosinofílica tenía más síntomas, mayor obstrucción basal y mayor sensibilidad bronquial al salino hipertónico. El valor de FE_{NO} de 21 ppb fue el punto de corte que mejor se ajustaba a la eosinofilia en esputo del 3%. Este indicador se comportaba mejor entre los pacientes sin tratamiento esteroideo (sensibilidad 97%, especificidad 58%, VPP 86%, VPN 88%) que entre los que recibían corticosteroides (sensibilidad 81%, especificidad 25%, VPP 74%, VPN 33%).

Conclusión: Los valores de FE_{NO} ≥ 21 ppb se asocian a eosinofilia en esputo. En sujetos que no reciben tratamiento esteroideo, valores de FE_{NO} < 21 ppb descartan eosinofilia bronquial.

Palabras clave: Asma. Punto de corte óxido nítrico. Fenotipo eosinofílico. Curvas ROC. Eosinófilos en esputo.

Introduction

Identification of an asthma phenotype has important implications. The eosinophilic phenotype (sputum eosinophils $\geq 3\%$) identifies patients who will show a good response to corticosteroids [1] and T_H2 immunomodulators [2]. The superiority of sputum eosinophils over asthma guidelines in tailoring corticosteroids has been confirmed by a meta-analysis [3]. However, induced sputum is far from becoming widely used, since it requires highly trained personnel, collaboration from patients, and individualized evaluation of samples. In addition, results are not available immediately.

Fractional exhaled nitric oxide (FeNO) correlates with the sputum eosinophil count. Portable devices for measuring FeNO are fast, cheap, and relatively easy to use. The American Thoracic Society recommends measurement of FeNO for detection of eosinophilic airway inflammation and prediction of the likelihood of response to inhaled corticosteroids (ICSs) [4]. In addition, they established cut points of 50 ppb (35 ppb in children) and recommend caution when values of 25-50 ppb (20-35 ppb in children) are detected. However, tailoring asthma treatment based on FeNO levels has not been effective in improving asthma outcomes in children and adults [3], probably owing to the lack of specificity of FeNO for eosinophilic inflammation, but also to the different cut points applied. We used a portable device to determine the FeNO cut point that best fits $\geq 3\%$ sputum eosinophils in asthma patients.

Patients and Methods

The study population comprised 129 consecutive, nonsmoking asthma outpatients who gave their written

informed consent to participate in this cross-sectional study (January 2010 to January 2011) (Table 1). Asthma was diagnosed according to the GEMA Asthma Guidelines [5]. Patients completed the Asthma Control Test (ACT) questionnaire [6]. FeNO was measured using a portable handheld electrochemical device (NIOX MINO, Aerocrine AB). From total lung capacity, patients exhaled for 10 seconds at 50 mL/s.

On the same day, sputum was obtained as described elsewhere [7]. Hypertonic saline 5% was given for 3 periods of 10 minutes. Spirometry (MasterScope PC, Jaeger) was performed at baseline and after each period. The slope of the dose-response curves (rate between maximal fall in forced expiratory volume in 1 second [FEV₁] and baseline FEV₁) defined bronchial sensitivity to hypertonic saline. Decreases in FEV₁ $\geq 15\%$ from baseline were considered significant.

Statistical Analysis

Patients were classified as having noneosinophilic asthma ($< 3\%$ sputum eosinophils) and eosinophilic asthma ($\geq 3\%$). The Mann-Whitney test and Spearman correlation coefficient were used. Receiver operating characteristic (ROC) curves were constructed to find the FeNO cut point that best classified eosinophilic asthma.

We calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for several FeNO cut points. Logistic regression analysis was applied to assess the relationship between the binary outcome (eosinophils $< 3\%$ or $\geq 3\%$) and the study variables. We analyzed the complete sample and stratified patients according to use of ICSs.

Table 1. Characteristics of Study Patients by Sputum Eosinophil Count^a

	Total N=129	Noneosinophilic n=28	Eosinophilic n=97	P Value
Age, y	26.4 (16.8)	25.1 (15.8)	26.7 (17.1)	.810
Weight, kg	55.3 (17.2)	43.7 (9.8)	56.4 (17.0)	.220
Male gender, No. (%)	59 (42.2%)	18 (64.3%)	41 (42.2%)	.065
Atopy, No. (%)	101 (78.3%)	21 (75%)	80 (82%)	.633
Polyposis, No. (%)	14 (10.8%)	3 (10.7%)	11 (11.3%)	.803
ICSs, No. (%)	44 (34.1%)	10 (35.7%)	34 (35%)	.839
FEV ₁ , No. (%)	106.9 (18.9)	109.8 (23.3)	106.2 (17.7)	.524
FEV ₁ /FVC, %	77.2 (9.6)	80.4 (10.7)	76.2 (9.1)	.010 **
Hypertonic saline-DRS	5.08 (9.7)	3.0 (5.5)	6.8 (7.3)	.003 **
Sputum total cell count, No.	2595 (1956)	2000 (1356)	2781 (2081)	.130
Sputum eosinophils, %	8.4 (10.0)	1.8 (2.1)	10.2 (10.1)	<.001 **
Sputum neutrophils, %	37.8 (23.0)	27.4 (19.7)	40.6 (23.1)	.006 **
FeNO	55.6 (45.9)	38.8 (44.1)	60.2 (45.5)	<.001 **
ACT	21.6 (3.6)	23.0 (2.7)	21.2 (3.7)	.011 *

Abbreviations: ACT, Asthma Control Test; DRS, dose-response curve; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ICSs, inhaled corticosteroids.

^aValues are expressed as mean (SD) unless otherwise indicated.

Results

Table 1 displays the results for the variables recorded. Response to osmotic stimulus (dose-response slope of the curve) correlated with sputum eosinophilia (0.46, $P < .001$) and FeNO (0.46, $P < .001$). Two-thirds of the patients were classified as having the eosinophilic phenotype. Compared with patients who had the noneosinophilic phenotype, eosinophilic asthmatics had a lower FEV₁/forced vital capacity (FVC), higher sensitivity to hypertonic saline, and poorer asthma control. They also had higher levels of FeNO and sputum neutrophils. FeNO performed well overall, with a total area under the ROC curve of 0.79, which increased in corticosteroid-naïve patients (0.81) and decreased in patients treated with ICSs (0.73) (Figure).

The FeNO cutoff value of 21 ppb was the best fit to 3% sputum eosinophilia (sensitivity, 91%; specificity, 47%; PPV, 82%; NPV, 68%). This 21-ppb cut point performed better in corticosteroid-naïve asthmatics than among those on ICSs (sensitivity, 97% vs 81%; specificity, 58% vs 25%; PPV, 86% vs 74%; NPV, 88% vs 33%). Data regarding the other FeNO cut points tested are shown in Table 2.

The logistic regression models showed that FeNO was the only variable to be significantly associated with eosinophilia $\geq 3\%$ (0.60, $P < .001$). The inclusion of the remaining variables (sex, atopy, and ICSs) neither improved the predictive capacity of the model nor changed the estimated coefficients for FeNO.

Discussion

Even in the absence of asthma [8], ongoing bronchial inflammation is a risk factor for lung damage, decline in FEV₁, asthma exacerbations, and fixed airway obstruction [9]. In our study, eosinophilic asthmatics had more symptoms, more airway obstruction, and higher sensitivity to osmotic stimulus. The absence of differences in the use of ICSs between the groups indicates that any such differences would not likely be due to undertreatment with anti-inflammatory agents.

The identification of a pattern of bronchial eosinophilic inflammation helps to decide when to initiate or increase treatment with ICSs or T_H2 immunomodulators [10]. Induced sputum could be considered the gold standard, but it is time-

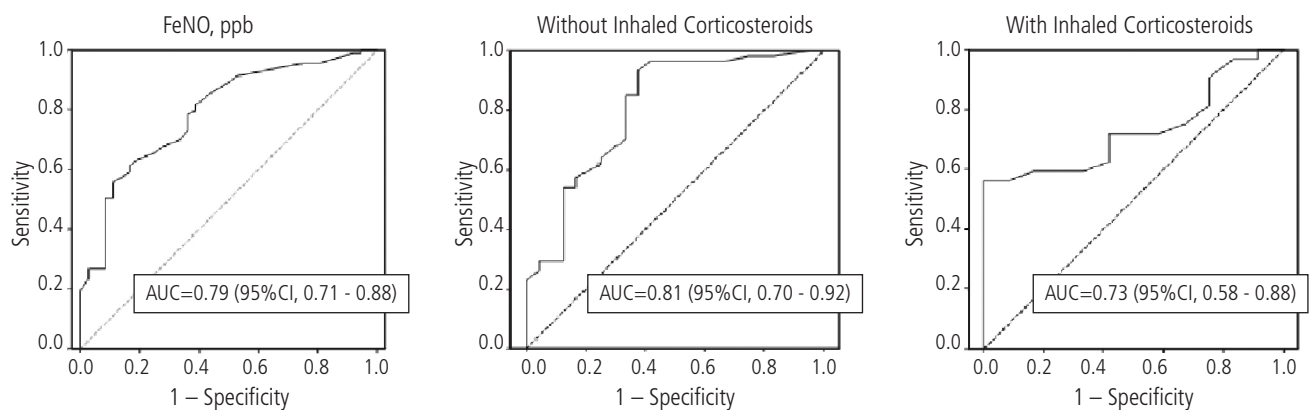


Figure. Receiver operating characteristic curves of FeNO to identify a sputum eosinophil count $\geq 3\%$ in all study patients and according to use of inhaled corticosteroids. FeNO indicates fractional exhaled nitric oxide.

Table 2. Data for the FeNO Cut Points Tested to Predict a Sputum Eosinophil Count $\geq 3\%$ in Nonsmoking Asthma Patients

FeNO, ppb	All Patients				ICSs				No ICSs			
	Se, %	Sp, %	PPV, %	NPV, %	Se, %	Sp, %	PPV, %	NPV, %	Se, %	Sp, %	PPV, %	NPV, %
17	96	19	75	64	91	25	76	50	98	17	75	80
21	91	47	82	68	81	25	74	33	97	58	86	88
26	82	61	84	56	72	58	82	44	87	62	86	65
31	73	64	84	48	63	58	80	37	79	67	86	55
36	66	75	87	46	56	92	95	44	71	67	84	47
41	60	83	90	45	56	100	100	46	62	75	86	44

Abbreviations: FeNO, fractional exhaled nitric oxide; ICSs, inhaled corticosteroids; NPV, negative predictive value; ppb, parts per billion; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

consuming and not always available. Moreover, although inhalation of hypertonic saline aids expectoration, a number of asthma patients are not able to produce sputum suitable for evaluation. The bronchial response to inhalation of hypertonic saline indirectly mirrors airway inflammation [11]. Accordingly, we observed a higher degree of bronchial sensitivity to such agents in eosinophilic asthmatic patients. However, this test is also time-consuming and does not provide a threshold value for eosinophilic bronchial inflammation.

Measurement of FeNO in exhaled air is a noninvasive and standardized method of evaluating inflammation [4]. Portable FeNO analyzers generate reproducible and immediate results relatively easily and inexpensively. The American Thoracic Society recommends evaluation of FeNO levels in asthma monitoring and considers that adults with FeNO values ≥ 50 ppb are very likely to respond to corticosteroids, whereas those with FeNO values ≤ 25 ppb would barely respond to anti-inflammatory treatment [4]. They also recommend careful evaluation of patients with FeNO values ranging from 25 ppb to 50 ppb [4]. These midrange values become particularly relevant in asthma patients, many of whom are taking anti-inflammatory treatment and have other conditions such as atopy, rhinitis, nasal polyps, obesity, and anxiety, all of which affect FeNO levels [4]. Identification of patients with eosinophilic airway inflammation in particular is clinically relevant when attempting to prevent undertreatment and overtreatment. In our clinical setting, we observed that a FeNO cut point of 21 ppb was the best fit for 3% sputum eosinophils. In contrast, Berry et al [12] reported a cut point of 8.3 ppb. In our opinion, such differences could be due to methodology, since Berry et al used a stationary chemiluminescence analyzer and measured FeNO at 250 mL/s [12]. At 50 mL/s, their cut point increased to 36 ppb.

Specificity and NPV increased in ICS-naïve patients, suggesting that FeNO values ≥ 21 ppb are highly suggestive of abnormal sputum eosinophilia and that treatment with ICSs should be recommended. However, it is remarkable that the sensitivity, specificity, and predictive values of a FeNO cut point ≥ 21 ppb worsened among patients on ICSs. By lowering the cut point, the NPV improved to 50%, but specificity remained low. These data suggest that both FeNO and sputum eosinophils respond differently to ICSs. Studies performed with immunomodulatory drugs in severe asthma show that anti-IL-5 reduces eosinophilia (but not FeNO) and the frequency of asthma exacerbations [13], whereas dupilumab, which prevents the activity of both IL-4 and IL-13, decreases the frequency of asthma exacerbations and reduces the values of FeNO and other inflammatory markers. However, it does not considerably change peripheral blood eosinophilia [14]. Consequently, it has been suggested that FeNO would be more accurately defined as a marker of T_H2 -mediated inflammation, which also includes airway eosinophilia, but not as a marker of eosinophilic inflammation per se.

We are aware that these markers cannot be used interchangeably, given that they do not respond identically to treatment. Nevertheless, in our opinion, this observation could be due to the range of different asthma phenotypes, which were not identified separately in the present study. Whereas bronchial eosinophilia is always abnormal, the FeNO value

is not a specific marker of eosinophilic inflammation, since FeNO is also synthesized in large amounts by epithelial cells and its values are affected by many factors and diseases (eg, nasal polyps, allergic rhinitis, and viral infections). Therefore, in our opinion FeNO should only be considered an indirect marker of eosinophilic bronchial inflammation.

Other limitations of our study include the fact that we did not take into account or homogenize measures of FeNO for factors that could modify the results (eg, height, weight, body mass index, and genetic background). However, a recent report on more than 13 000 individuals showed that despite the large individual variation in FeNO values, these and other individual factors affected FeNO little in absolute terms [15]. The authors did not consider it necessary to provide separate reference values for these variables. Furthermore, we cannot be confident of patients' adherence to ICSs, since we did not assess this variable. However, our study was based on the daily practice of treating patients with asthma attending an allergy clinic. Therefore, we conclude that in our clinical setting, a threshold FeNO value of ≤ 21 ppb in patients not taking corticosteroids very likely rules out airway eosinophilia and points to the need to establish other explanations for symptoms. Our results also suggest that each laboratory should undertake studies to evaluate which FeNO value identifies abnormal sputum eosinophils and persistent airway eosinophilia.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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