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Can Fractional Exhaled Nitric Oxide with Blood Eosinophil Count Have a Place in the Diagnostic Algorithm for Asthma?

İlkay KOCA KALKAN[1], Gözde KÖYCÜ BUHARİ[1], Hale ATEŞ[1], Buket BAŞA AKDOĞAN[1], Özlem ÖZDEDEOĞLU[1], Kurtuluş AKSU[1], Ferda Öner ERKEKOL[2]

[1] Department of Allergy and Clinical Immunology, University of Health Sciences, Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital, Ankara, Turkey
[2] Department of Chest Diseases, Division of Immunology and Allergy, Ankara Yıldırım Beyazıt University School of Medicine, Ankara, Turkey

Corresponding Author: İlkay Koca Kalkan ✉ ilkay.koca@gmail.com

ABSTRACT

Objective: Guidelines suggest using bronchial provocation testing (BPT), which is hard to attain, in patients with asthma-like symptoms presenting with nondiagnostic spirometric tests. To eliminate the risk of over/underdiagnosing asthma, we aimed to evaluate the predictive value of not only fractional exhaled nitric oxide (FeNO) but also other easily accessible clinical indices for ruling in/out asthma.

Materials and Methods: This retrospective study included adults presenting to our clinic with respiratory symptoms suggestive of asthma but with normal spirometric values and negative reversibility test, who underwent FeNO and methacholine BPT (MchBPT). Medical records were used to obtain descriptive characteristics, clinical history, allergy screening, eosinophils in peripheral blood, and spirometry.

Results: Among 51 patients, 19 were diagnosed with asthma. Body mass index and blood eosinophils were significantly higher in patients with positive MchBPT (p=0.042 and p=0.037, respectively). No significant difference was found in other indices, including FeNO (p=0.293). Receiver operating characteristic curve analysis revealed the best diagnostic cutoff level for FeNO as 14 ppb and blood eosinophil as 150/µl for the prediction of positive MchBPT (with 63.16%-62.5% and 80%-61% sensitivity-specificity, respectively). These two indices were the only independent predictors of positive BHR, and the model of FeNO>14ppb combined with eos>150/µl showed 100% specificity with a 100% negative predictive value.

Conclusion: Our results suggest using the combination of FeNO with blood eosinophil count as a rule-out test, adding a new step in the algorithmic diagnosis of asthma. This might avoid an unnecessary BPT procedure, reduce the risk of over/under-diagnosis of asthma, and hasten the correct diagnosis.

Keywords: Rule-out test, bronchial provocation testing, methacholine, spirometry, cut-off values

INTRODUCTION

Asthma is a common, heterogeneous disease with chronic airway inflammation, usually associated with airway hyperresponsiveness to direct or indirect stimuli. These features usually persist, even in the absence of symptoms and abnormal lung function. Asthma diagnosis is based on the history of distinctive symptom patterns, including breathlessness, wheezing, chest tightness, and cough, with evidence of variable airflow limitation (1). Measurement of lung function is mensuration at a certain point and therefore, if patients are asymptomatic during the day, especially in mild asthma, airway obstruction is usually absent pending a study with spirometry, hence leading to diagnostic uncertainty (2). At this point, since airway hyperresponsiveness and airway inflammation are likely to persist even without obvious respiratory symptoms on a given day, bronchial provocation for determining

ORCID İlkay Koca Kalkan / 0000-0002-9788-1056, Gözde Köycü Buhari / 0000-0002-0787-0370, Hale Ateş / 0000-0001-8374-5814, Buket Başa Akdoğan / 0000-0003-2919-6660, Özlem Özdedeoğlu 0000-0001-8421-9904, Kurtuluş Aksu / 0000-0001-6195-1158, Ferda Öner Erkekol / 0000-0001-9155-0304

bronchial hyperresponsiveness remains as a reference standard in case of inconclusive spirometry results (3). However, bronchial provocation is generally only available at certain lung function laboratories, requires specialized equipment with professional staff, is time-consuming and cost-intensive, can be technically challenging for patients, and also has the risk of severe bronchospasm (4).

On the other hand, the fractional exhaled breath nitric oxide (FeNO) concentration in exhaled air is a non-invasive point of care test for estimating type-2 (T_2) airway inflammation in the lung (5). Since the first scientific publication on the application of FeNO in asthma in 1993, the technique became more common with the use of new portable hand-held devices available in hospitals, clinics and private offices (6). FeNO has the advantage of being standardized, non-invasive, quick, simple and easy to reproduce. The FeNO level was found to be related to airway hyperresponsiveness as well as to bronchial eosinophilic inflammation (7). Asthmatic patients have been shown to produce higher FENO levels, even in milder stages of the disease, in correlation with the inflammation in airway epithelium (5). However, asthma is a heterogeneous disease, not only related to NO but also many other inflammatory pathways. In addition, different FeNO cut-off values recommended in various studies in the literature have only moderate sensitivities and specificities. So, their use would lead to many false positives or false negatives.

Based on these facts, the purpose of our study was to evaluate the predictive value of not only FeNO but also other clinical indices, at ruling in or out asthma in patients with normal spirometry and a suggestive history without bronchodilator response. We mainly aimed to focus on the combined effect of these parameters in clinical prediction models which can be applied as rule-in or rule-out tests in asthma diagnosis in the primary care setting.

MATERIALS and METHODS

Study Design and Subject Selection

A retrospective, single-center study was performed with the data of the patients who presented to our allergy clinic with respiratory symptoms suggestive of asthma (cough, wheezing, dyspnea, chest tightness) showing normal spirometric values with negative bronchodilator testing to demonstrate reversibility after 400 mcg of salbutamol. The data was collected in 4 consecutive months. Patient charts were evaluated retrospectively and sequentially to get the demographic and clinical characteristics. The study was approved by the Ankara Keçiören Educational Research Hospital Clinical Research Ethics Committee (22.07.2020/2139). Informed consent was waived due to the retrospective nature of this study.

The study population consisted only of patients meeting all of the following criteria: age 18-65 years, symptoms suggestive of asthma (cough, wheezing, dyspnea, chest tightness), normal spirometry and no bronchodilator reversibility after 400 mcg of salbutamol inhalation, and in whom FeNO and methacholine provocation tests performed. Patients meeting the following criteria were excluded from the study: possible/definite diagnosis of other chronic pulmonary diseases (COPD, bronchiectasis, sarcoidosis, etc.), acute upper or lower respiratory tract infections within the previous 6 weeks, and a significant problems causing an inability to comply with the study tests.

The medical records were used to obtain descriptive characteristics and the clinical history. The results of thoracic imaging, allergy screening (skin prick test and/or specific IgE assays), total IgE level (TIgE), percentage and absolute cell count of eosinophils in peripheral blood, spirometry, FeNO, and methacholine provocation tests were reviewed and analyzed.

Patients were evaluated for each respiratory symptom suggestive of asthma (cough, wheezing, dyspnea, and chest tightness) with the visual analog scale (VAS).

Spirometry

Spirometric assessments were done with a spirometer (Zan 100, nSpire Health Inc., Oberthulba, Germany) according to the standards set by the ERS and ATS (8). Percentages of predicted values were based on sex, height, weight and age for the FEV1, FVC, $MEF_{50,}$ $MEF_{75,}$ MEF_{25-75} and PEF values. FEV_1/FVC is expressed as an absolute value. A reversibility test was performed by lung function measurement after 15 minutes of using 4 puffs of 100 mcg salbutamol. Significant reversibility was defined as an increase in FEV1 of >12% and >200 ml from the baseline (1).

Fractional Exhaled Nitric Oxide (FeNO)

FeNO was measured by using an exhaled nitric oxide analyzer (NIOX MINO, Aerocrine AB, Solna, Sweden)

at a standard flow rate of 50 ml/s, in accordance with ATS/ERS recommendations (9). FeNO was expressed in parts per billion (ppb). FeNO measurements were made on the same day, before the spirometric evaluations and methacholine provocation tests.

Methacholine Bronchial Provocation (MchBPT)

Methacholine bronchial provocation tests were performed in accordance with the ERS guideline for the five-breath dosimeter method (KoKo Digidoser; Quantum Research) (10). The patient inhaled a dose of isotonic saline, followed by 5 methacholine dilutions of 0.0625, 0.25, 1, 4 and 16 mg/ml, until the highest concentration of 16 mg/ml or a 20% decrease in FEV_1 was reached. A positive test result was defined by a decrease in FEV_1 20% or more. The provocative concentration of methacholine required to induce a 20% fall in FEV_1 (PC_{20}) was calculated in each subject with a positive test. The dose-response slope (DRS) was calculated for all subjects as the percent of reduction of FEV1 from the post-saline value to the value measured after the last methacholine dose administered divided by the final cumulative methacholine dose administered as defined by O'Connor (11).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 25 (SPSS, Chicago, IL) was used for the statistical analysis of this study. Descriptive statistics (frequencies, means and standard deviations, median and minimum-maximum) were calculated. The Kolmogorov-Smirnov test was used to assess the normality of data distribution. Normally distributed data are presented as mean±standard deviation. We used the methacholine bronchial provocation test as the gold standard for defining airway hyperresponsiveness. The univariate analyses to identify variables associated with the methacholine bronchial provocation test outcome was conducted using the Chi-square, Fisher exact, Student's t and Mann-Whitney U tests, where appropriate. For the multivariate analysis, the possible factors identified with univariate analysis were further entered into the binary logistic regression analysis to determine independent predictors of patient outcome. The capacity of single or combined measurements in predicting the presence of airway hyperresponsiveness was analyzed by constructing ROC (receiver operating characteristic) curves and measuring the area under the curves (AUC). The optimal value giving the highest sum of bronchial hyperreactivity diagnostic sensitivity and specificity was used as a cutoff value. Positive predictive values (PPV) and negative predictive values (NPV) were calculated for each cutoff value. Continuous test variables were converted to dichotomous state variables based on the cutoff values, and multiple logistic regression was performed to get a predictive equation. Subsequently, ROC curves were determined for the combined measurements with the dichotomous state variables. A p value <0.05 was considered statistically significant.

RESULTS

Clinical data from 51 patients with respiratory symptoms suggestive of asthma but with normal spirometric values and negative reversibility tests who had undergone FeNO and MchBPTs were included in this study. Nineteen (37.25%) patients were diagnosed as asthma with bronchial hyperreactivity. The median patient age was 40.2 (±12.3) years with 76.5% (n=39) females. In total, 27.6% (n = 14) had a positive skin prick test. Most demographic variables and clinical characteristics did not differ between the groups at baseline. However, BMI was significantly higher in patients with positive MchBPT (p = 0.042) (Table I). In addition, both the percentage and absolute blood eosinophil counts in the peripheral blood were significantly higher in patients with positive MchBPT (p = 0.014 and p = 0.037, respectively) (Table II). No significant differences were found in the other indices, including pulmonary function results. The level of FeNO in patients with positive BHR was higher compared with the non-BHR group, but the difference was not statistically significant (19.58±13.5 ppb vs. 16.34±8.3 ppb, p = 0.293).

The overall diagnostic utility of FeNO levels to distinguish BHR from non-BHR patients was studied by ROC curve analysis, which provided 14 ppb (AUC= 0.544 [95% CI, 0.365-0.722]) as the optimal cut-off value with the highest sum of sensitivity and specificity (Figure 1) (Table III). As the difference in absolute blood eosinophil counts between patients with or without BHR became statistically significant, demonstrating that this measurement might predict BHR status, the ROC curve was created to evaluate the prognostic value of this measurement (Figure 2). The AUC of eosinophil count for a positive MchBPT was 0.695 (95% CI, 0.517-0.874) with an optimal cutoff value of 150/μl with highest sum of sensitivity and specificity (Table III).

In the univariate analysis, which compared patient characteristics based on the FeNO>14ppb cutoff value,

Table I: Demographic and clinical characteristics of the study population.

	Total (n=51)	BPT		*p*
		+ (n=19)	- (n=32)	
Gender, n (%)				
Female	39 (76.5)	15 (78.9)	24 (75)	0.748
Male	12 (23.5)	4 (21.1)	8 (25)	
Age, y (±SD)	40.2 (±12.3)	41.37 (±12.86)	39.53 (±12.12)	0.611
BMI, kg/m^2 (±SD)	27.1 (±5.8)	29.28 (±4.71)	25.84 (±6.19)	**0.042**
Atopy presence, n (%)	14 (27.6)	5 (27.8)	9 (28.1)	0.979
Exposure to allergen, n (%)	5 (35.7)	1 (20)	4 (44.4)	0.580
Familial predisposition to asthma, n (%)	23 (45.11)	8 (42.1)	15 (46.9)	0.741
Smoking history, n (%)				
Never	36 (70.6)	13 (68.4)	23 (71.9)	0.884
Ex-smoker	12 (23.5)	5 (26.3)	7 (21.9)	
Current smoker	3 (5.9)	1 (5.3)	2 (6.3)	
Pack-years	4 (1-60)	2 (1-33)	6.5 (1-60)	0.399
Cough				
Median duration of symptoms, months	12 (0-300)	6 (0-300)	24 (0-120)	0.389
VAS, mm	42.6 (±27.9)	40.94 (±31.95)	43.71 (±25.51)	0.763
Wheezing				
Median duration of symptoms, months	5.5 (0-120)	6 (0-120)	2 (0-78)	0.668
VAS, mm	25.9 (±25.2)	34 (±33.91)	21.05 (±17.44)	0.163
Dyspnea				
Median duration of symptoms, months	30 (0-180)	30 (0-120)	30 (0-180)	1
VAS, mm	38.9 (±30.2)	42.43 (±31.56)	37 (±29.97)	0.595
Chest Tightness				
Median duration of symptoms, months	3 (0-120)	4 (0-120)	2 (0-120)	0.731
VAS, mm	15 (0-95)	30 (0-95)	15 (0-90)	0.411

BMI: Body mass index, **BPT:** Bronchial provocation test, **VAS:** Visual analog scale.

Table II: Spirometry and other laboratory results of the study population.

	Total (n=51)	BPT		*p*
		+ (n=19)	- (n=32)	
FEV_1, *% pred* (±SD)	99.69 (±13.81)	99.58 (±12.84)	99.75 (±14.55)	0.965
FVC, *% pred* (±SD)	104.3 (±14.81)	105.11 (±11.67)	103.94 (±14.35)	0.787
FEV_1/FVC (±SD)	82.55 (±7.35)	81.11 (±6.19)	81.81 (±8.04)	0.744
MEF_{50}, *% pred* (±SD)	89.2 (±27.2)	82.5 (±22.0)	93.2 (±29.4)	0.177
MEF_{75}, *% pred* (±SD)	90.9 (±20.0)	85.4 (±14.1)	94.2 (±22.4)	0.131
MEF_{25-75}, *% pred* (±SD)	82.71 (±25.74)	78.32 (±18.94)	85.31 (±29.01)	0.353
PEF, *% pred* (±SD)	86.59 (±18.56)	81.74 (±13.6)	89.47 (±20.61)	0.152
TIgE, median (min-max)	81.5(1.14-3860)	109 (3.94-1045)	46 (1.14-3860)	0.165
Eosinophil, % (±SD)	2.50 (±1.82)	3.66 (±2.39)	1.88 (±1.02)	**0.014**
cells /µl, median (min-max)	167 (10-1233)	276 (43-902)	125 (10-1233)	**0.037**
FeNO, *ppb* (±SD)	17.55 (±10.53)	19.58 (±13.50)	16.34 (±8.30)	0.293

FEV_1: Forced expiratory volume in 1 second, **FVC:** Forced vital capacity; **MEF_{50}:** Maximal expiratory flow at 50% of FVC, **MEF_{75}:** Maximal expiratory flow at 75% of FVC, **MEF_{25-75}:** Maximal expiratory flow at 25-75% of FVC, **PEF:** Peak expiratory flow, **%pred:** Percentages of predicted values, **TIgE:** Total immunoglobulin E.

the factors that have significant effects on FeNO were identified (duration and severity of wheezing, FEV_1/FVC, $MEF_{50,}$ $MEF_{75,}$ MEF_{25-75}, TIgE, FEV_1 drop during BPT and dose response slope) (Table IV).

A binary logistic regression analysis was performed to identify the independent factors related with positive BHR, with the defined borderline significant factors ($p<0.2$). As a result, FeNO>14ppb and Eos>150/μl were identified (Table V).

Using the cutoff values for FeNO>14ppb and Eos>150/μl the continuous test variables were converted to dichotomous variables. The ROC analyses were repeated using these 2 new measures (AUC of FeNO>14ppb was 0.688 [95% CI, 0.520-0.856] and AUC of Eos>150/μl 0.704 [95% CI, 0.541-0.866]). Then, logistic regression was performed to obtain a predictive model of the combination measure, to determine whether combining the measures will improve the BHR estimate. The AUC of the model FeNO>14ppb combined with Eos>150/μl was 0.792 (95% CI, 0.660-0.923), with a sensitivity and specificity of 100% and 46.3%, respectively. PPV and NPV were 48.5% and 100%, respectively (Figure 3).

DISCUSSION

In this study we evaluated the capability of FeNO and other noninvasive/easily accessible clinical measures to predict the presence of BHR among individuals reporting respiratory symptoms suggestive of asthma, but with normal spirometric values and negative reversibility tests. Our data showed that only blood eosinophil and BMI were statistically higher in these subjects with BHR. Neither FeNO, which was higher in the positive BHR group, nor other clinical indices reached statistical significance. ROC

Table III: Optimal cutoff values for the prediction of positive bronchial provocation.

Parameter	Cutoff	AUC (95%CI)	Sensitivity	Specificity	PPV (%)	NPV (%)	*p*
FeNO, ppb	14	0.544 (0.365-0.722)	63.16	62.5	50	74.1	0.606
Eos, /μl	150	0.695 (0.517-0.874)	80	61	52.5	85	0.037

AUC: Area under the curve; **Eos:** Eosinophils; **NPV:** Negative predictive value; **PPV:** Positive predictive value.
*The cutoff points were obtained by maximizing the sum of sensitivity and specificity (maximum Youden index).

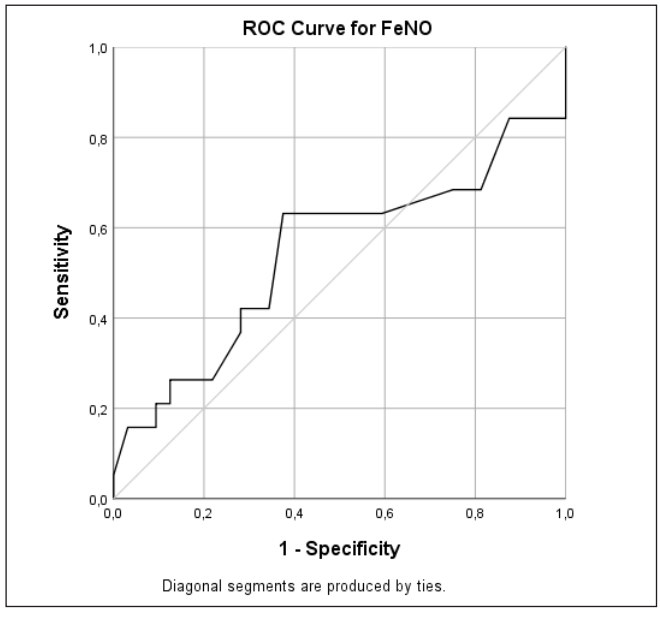

Figure 1. Receiver-operating characteristic (ROC) curve analysis of the diagnostic sensitivity and specificity of FeNO for the diagnosis of asthma.

AUC: Area under the curve, **NPV:** Negative predictive value, **PPV:** Positive predictive value.

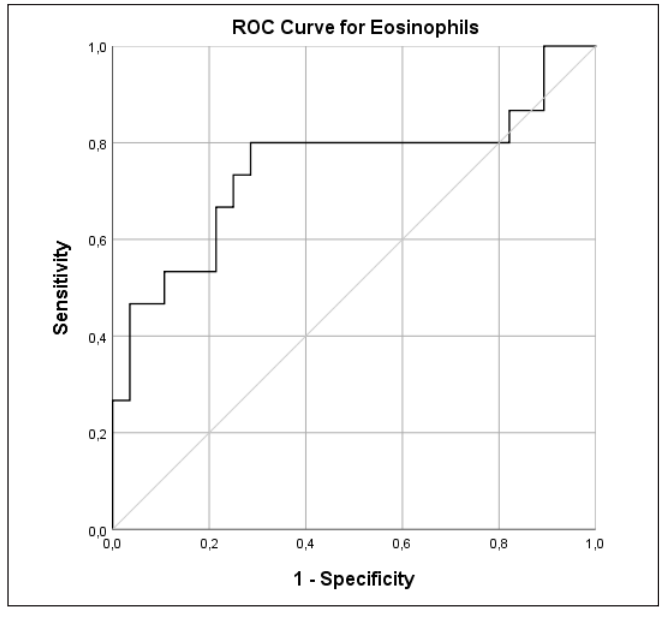

Figure 2. Receiver-operating characteristic (ROC) curve analysis of the diagnostic sensitivity and specificity of eosinophils for the diagnosis of asthma.

AUC: Area under the curve, **NPV:** Negative predictive value, **PPV:** Positive predictive value.

Table IV: Demographic and clinical characteristics of the subjects according to their FeNO levels.

	FeNO >14 ppb (n=24)	FeNO ≤14 ppb (n=27)	
Gender (F), n (%)	13 (54)	26 (96)	**<0.001**
Age, y (±SD)	41.37 (±12.9)	39 (±11.8)	0.488
BMI, kg/m² (±SD)	28.3 (±4.2)	26 (±6.9)	0.159
Atopy presence, n (%)	8 (33.3)	6 (23.1)	0.420
Cough			
Median duration of symptoms, months	12 (0-300)	4.5 (0-300)	0.339
VAS, mm	49 (±28.5)	37.5 (±26.8)	0.193
Wheezing			
Median duration of symptoms, months	18 (0-120)	1.5 (0-60)	**0.014**
VAS, mm	41 (±27.8)	14.1 (±15.2)	**0.002**
Dyspnea			
Median duration of symptoms, months	36 (0-120)	18 (0-180)	0.205
VAS, mm	39.8 (±31.0)	38 (±30.2)	0.854
Chest Tightness			
Median duration of symptoms, months	5 (0-120)	1 (0-120)	0.358
VAS, mm	20 (0-95)	0 (0-90)	0.339
FEV_1, *% pred* (±SD)	99.6 (±11.5)	99.7 (±15.7)	0.992
FVC, *% pred* (±SD)	107.5 (±11.5)	101.5 (±16.7)	0.144
FEV_1/FVC (±SD)	77.9 (±6.8)	84.7 (±6.3)	**0.001**
MEF_{50}, *% pred* (±SD)	79.4 (±23.6)	97.9 (±27.5)	**0.014**
MEF_{75}, *% pred* (±SD)	84.8 (±17.6)	96.3 (±20.8)	**0.039**
MEF_{25-75}, *% pred* (±SD)	74 (±22.4)	90.3 (±26.4)	**0.023**
PEF, *% pred* (±SD)	81.9 (±17.6)	90.7 (±18.7)	0.090
TIgE, median (min-max)	120 (1.3-3860)	24.8 (1.14-864)	**0.005**
Eosinophil, % (±SD)	2.8 (±2.2)	2.2 (±1.2)	0.289
cells /µl, median (min-max)	212 (10-1233)	132.5 (44-507)	0.189
BPT positivity, n (%)	12 (50)	7 (25.9)	0.076
Methacholine pC20 (mg/ml)	4.4 (0-14.9)	8.5 (0.5- 15)	0.285
FEV_1 drop during BPT			
lt	0.57 (±0.39)	0.32 (±0.28)	**0.013**
%	19.1 (±12.4)	11.4 (±9.1)	**0.014**
Dose Response Slope	0.88 (0.15-41.99)	0.54 (-.78-11.20)	**0.031**

F: Female, **SD:** Standard deviation, **VAS:** Visual analog scale, **FEV_1:** Forced expiratory volume in 1 second, **FVC:** Forced vital capacity, **MEF_{50}:** Maximal expiratory flow at 50% of FVC, **MEF_{75}:** Maximal expiratory flow at 75% of FVC, **MEF_{25-75}:** Maximal expiratory flow at 25-75% of FVC, **PEF:** Peak expiratory flow, **%pred:** Percentages of predicted values, **TIgE:** Total immunoglobulin E.

Table V: Binary logistic regression analysis results to predict independent factors for airway hyperresponsiveness.

	OR	*p*	95% CI
BMI	1.189	0.292	0.861-1.641
MEF_{50}%*pred.*	1.038	0.349	0.960-1.121
MEF_{75}%*pred.*	1.069	0.576	0.847-1.349
PEF%*pred.*	0.832	0.155	0.646-1.072
TIgE	0.998	0.123	0.996-1.000
Eosinophil >150/µl	16.467	**0.040**	1.142-237.551
FeNO> 14ppb	26.393	**0.035**	1.268-549.479

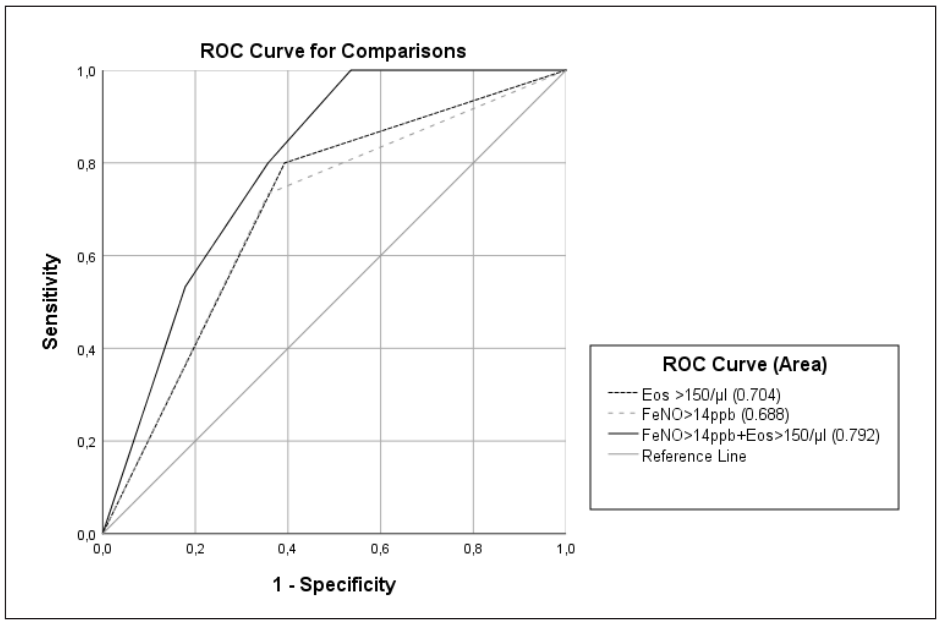

Figure 3. ROC curves of dichotomous state variables of FeNO combined with eosinophils in predicting positive bronchial provocation, compared with FeNO and eosinophils alone. Dichotomous state variables were separated according to cutoff values of FeNO and eosinophils.

Parameter	AUC (95%CI)	Sensitivity	Specificity	PPV (%)	NPV (%)	*p*
FeNO>14ppb	0.688 (0.520-0.856)	73.3	64.3	50	74.1	0.044
Eosinophil >150/µl	0.704 (0.541-0.866)	80	60.7	54.5	85.7	0.029
FeNO>14ppb+Eosinophil >150/µl	0.792 (0.660-0.923)	100	46.3	48.5	100	0.002

curve analysis demonstrated that the best diagnostic cutoff level for FeNO was 14 ppb, with 63.16% sensitivity and 62.5% specificity, and for blood eosinophil 150/µl, with 80% sensitivity and 61% specificity, for the prediction of positive MchBPT. Also, these two indices were the only independent predictors of a positive BHR.

The main finding in the present study is that when faced with a patient with respiratory symptoms suggestive of asthma and normal baseline spirometry, combination of FeNO >14ppb together with blood eosinophil >150/µl predicted BHR with a sensitivity and NPV of 100%. To the best of our knowledge, this is the first study in the English literature providing evidence for the use of combined specific cut-off levels for FeNO levels with blood eosinophil counts as a rule-out test in an algorithmic approach for asthma diagnosis with the highest certainty.

Asthma is a prevalent chronic disorder affecting around 300 million people worldwide, and it is likely that by 2025 a further 100 million may be affected (12). However, asthma misdiagnosis appears to be widespread, as both over- and under-diagnosis. It has been reported that about 1/3 of the cases were over-diagnosed, while the estimates of under-diagnosis vary between 19% and 73% (13-15). Both possibilities carry costs for the health of the patient and the healthcare system. Over-diagnosing leads to a delay in making an alternative diagnosis, which may be life threatening, and long-term overtreatment with unnecessary medicines carrying potential side-effects and financial costs. Under-diagnosis risks daily symptoms, productivity, quality of life, exacerbations, and long-term airway remodeling (15). Because of these reasons, the Global Initiative for Asthma (GINA) guidelines suggest objective documentation of the evidence on which an asthma diagnosis has been made before treating asthma (1). Both GINA and the British Thoracic Society (BTS) guidelines recommend the use of bronchial provocation testing when asthma is suspected in patients with a nondiagnostic spirometry/peak flow and reversibility test (1, 16). Such testing, however, requires sophisticated equipment and adequate health-care resources, which makes it only available in specialist centers. In addition, the test is time-consuming, thus placing a high demand on health-care resources causing long appointment lists. Some patients may also find it unpleasant and it therefore cannot be widely used to evaluate patients suspected of having asthma as it should be. Consequently, the risk of over- or under-diagnosing is increased. Therefore, the existence of

easily accessible rule-out or rule-in tests that will facilitate triage in a prospectively diagnostic algorithmic manner is important.

In many studies, it has been found that airway hyperresponsiveness correlates with airway inflammation, primarily with eosinophils, as assessed directly by induced sputum cell counts and bronchoalveolar lavage (BAL) or indirectly by blood eosinophils and FeNO (17-20). In the present study, we observed from indirect biomarkers that only blood eosinophil was correlated with airway hyperresponsiveness, not FeNO. Although the American Thoracic Society (ATS), the National Institute for Health and Care Excellence (NICE), and the British Thoracic Society (BTS) recommend FeNO measurement to guide the diagnosis and treatment of eosinophilic asthma, the 2020 GINA strategy report states that FeNO has not been established as useful for ruling in or ruling out a diagnosis of asthma (1, 16, 21, 22). This is probably because there are several FeNO cutoff points provided with a range of sensitivity and specificity for the diagnosis of asthma. Thereof, an optimal FeNO cutoff value for asthma could not be obtained. A recent review with a total of 13747 patients showed that, in adults, using FeNO cutoffs of less than 20, 20 to 29, 30 to 39, and 40 or more ppb, FeNO testing had sensitivities of 0.80, 0.69, 0.53, and 0.41 and specificities of 0.64, 0.78, 0.85, and 0.93, respectively (23). A very recent 2020 report from the National Asthma Education and Prevention Program Coordinating Committee Expert Panel Working Group reported that in corticosteroid-naive individuals with asthma, a FENO level below 20 ppb is most accurate for ruling out the asthma diagnosis with a sensitivity of 0.79 and a specificity of 0.77, which is still low. It also stated that specificity/sensitivity depends on the clinical situation and FENO test results should not be used alone to diagnose asthma (24). In the present study, we observed that the best diagnostic cutoff level for FeNO was 14 ppb, with 63.16% sensitivity and 62.5% specificity. Our study showed a lower optimal cutoff point, which may be explained by the fact that most of our patients had a milder form of the disease and a lower rate of atopy. For all of these reasons, especially in a real-life setting where milder forms of the disease are more prevalent as in our study population, the predictive value of FeNO as a rule-in or rule-out test for in asthma diagnosis is not fully satisfying and should be improved.

In this study, we found that the blood eosinophil count was statistically higher in subjects with BHR. So, ROC curve analysis was performed and demonstrated that the best diagnostic cutoff level for blood eosinophil count was 150/μl, with 80% sensitivity and 61% specificity, for the prediction of positive MchBPT. We observed that, although both blood eosinophil count and FeNO concentration were associated with eosinophilic airway inflammation, they demonstrated no significant correlation. This is because they reflect the different parts of the T helper 2 cell-driven inflammation, proven by their responsiveness to different biologic therapies for asthma. Treatment with mepolizumab (anti-interleukin-5) lowered blood eosinophil counts without affecting FeNO concentrations, while treatment with lebrikizumab (anti-interleukin-13) and dupilumab (anti-IL-4 and 13) reduced FeNO concentrations without affecting blood eosinophil counts (25-27). Therefore, it has been suggested that they can be used as complementary biomarkers of T helper 2 cell-driven inflammation. However, studies on combining these two biomarkers are still limited to their adequacy in predicting asthma control, wheeze, bronchial hyperresponsiveness, impaired lung function, exacerbations; and has not been investigated as a rule-out test in diagnosing asthma (28-31). By using the combined AUC curve model, we showed that the combination of FeNO and blood eosinophil count has a better sensitivity and negative predictive value for BHR then either one alone. Among individuals reporting respiratory symptoms with normal baseline spirometry, the use of these two biomarkers with the given cutoff values seemed to rule out asthma with a negative predictive value of 100%. For this reason, our findings suggest that the diagnostic pathway may begin with FeNO measurement and blood eosinophil count in clinical practice. Bronchial provocation would be redundant when FeNO and blood eosinophil count stay under a distinct cut-off value, with a meaningful NPV. When FeNO value and blood eosinophil count is higher, referral for bronchial provocation will be required. A positive response during bronchial provocation will aid decision making in the diagnosis of asthma, and a negative bronchial provocation response will rule out the disease. Within the limits of the data, our results show that FeNO concentration and blood eosinophil count are simple complementary measures, which may obviate the need for BPT in some patients when used as a composite biomarker.

Previous data confirm that direct airway responsiveness testing with methacholine or histamine is highly sensitive with a PC_{20} greater than 16 mg/mL for excluding the diagnosis of asthma with reasonable certainty, especially

in individuals with current asthma symptoms like our patients (32). However, this cut off point lacks specificity in healthy individuals because of false-positive challenges. In their study, Cockcroft et al. showed that in a group of young adults with no history or symptoms of asthma, 4.5% had a positive test result at a cut-point of 8 mg/ml and 21% at a cut-point of 16 mg/ml (33). These results indicate, as would be expected, that increasing the PC_{20} cut point increases the diagnostic sensitivity while reducing the diagnostic specificity, so every individual should be interpreted with caution according to their circumstances.

The present study has some limitations. First, the biomarkers used are both known markers of type 2 inflammatory response, and therefore other inflammatory patterns, such as neutrophilic inflammation, would be expected to be not identified. However, the enzyme inducible nitric oxide synthase (NOS2), which catalyzes the generation of NO, was shown to be induced in human airway epithelial cells not only by type 2 cytokines but also by the type 1 cytokine IFN-γ (34, 35). So, a very low FeNO value below 14 ppb might indicate the absence of airway inflammatory processes, both type 1 and type 2. Second, the study is limited by the small sample size and retrospective design, and the results of this study need to be confirmed in a prospective study with a larger patient population.

CONCLUSION

It seems worth the effort to optimize the strategies for algorithmically diagnosing asthma, especially for patients for whom BPT is not directly accessible. Our results suggest using the combination of determined optimal cut-off values for FeNO with blood eosinophil count as a rule-out test might be a way to avoid unnecessary BPT procedures, to reduce the risk of over/under diagnosis of asthma, and to hasten the correct diagnosis. With this respect, our results point towards the necessity of a confirmatory diagnostic study in terms of a triage test for partial replacement of bronchial provocation.

CONFLICT of INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

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