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Title: Can exhaled nitric oxide be a surrogate marker of bronchial hyperresponsiveness to adenosine 5'-monophosphate in steroid-naive asthmatic children?

Running Title: Exhaled Nitric Oxide Predicts Bronchial Hyperresponsiveness

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Abstract

Background: The interrelation between airway inflammation, bronchial hyperresponsiveness (BHR) and atopy remains controversial.

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Objective: The aim of this study is to document whether eNO may be used as a surrogate marker that predicts bronchial hyperresponsiveness (BHR) to adenosine 5'-monophosphate (AMP) in steroid-naïve school children with asthma.

Methods: This study was a retrospective analysis of steroid-naïve school age children with atopic and non-atopic asthma. All patients whose exhaled nitric oxide (eNO) levels had been measured and who had been challenged with both methacholine (MCH) and AMP were included. ROC analysis was performed, in both the atopic and the non-atopic groups, to evaluate the ability of eNO to detect the BHR to AMP.

Results: One-hundred and sixteen patients, sixty nine (59.5 %) of whom had been atopic, were included in the analysis. In the atopic group, eNO values were significantly higher in patients with BHR to AMP compared to those without BHR to AMP (51.9 ± 16.9 ppb vs 33.7 ± 16.4 ppb; $p < 0.001$), whereas in the non-atopic group the differences were not statistically significant (29.7 ± 16.9 ppb vs 22.6 ± 8.1 ppb; $p = 0.152$). In the atopic group, eNO levels ($R^2: 0.401$; $\beta: 0.092$; 95%CI: 1.19-14.42; OR: 7.12; $p = 0.008$) were found to be the only independent factor for BHR to AMP, whereas none of the parameters predicted BHR to AMP in the non-atopic group. The best cut-off value of eNO that significantly predicts BHR to AMP was 33.3 ppb in the atopic group ($p < 0.001$) whereas a significant cut-off value for eNO that predicts BHR to AMP were not determined in the non-atopic group ($p = 0.142$). An $eNO \leq 17.4$ ppb has 100% NPV and 100% sensitivity and 60.47% PPV for prediction of BHR to AMP in the atopic group.

Conclusions: Exhaled NO may be used to predict BHR to AMP in atopic but not in non-atopic steroid naïve asthmatic children.

INTRODUCTION

Bronchial hyperresponsiveness (BHR) is a hallmark of asthma and a consequence of the underlying airway inflammation[1]. BHR is evaluated using direct and/or indirect bronchial challenge tests (BCTs), that frequently utilize methacholine (MCH) and/or adenosine 5'-monophosphate (AMP) as bronchial stimulants which mediates bronchoconstriction by different mechanisms[2,3]. Previous studies have demonstrated that BHR to AMP may be a more direct indicator of airway inflammation than BHR to MCH and they indicated a more favorable relationship between "BHR to AMP" and atopy compared to "BHR to MCH" and atopy[4-8]. Thus, in the light of those evidences, BHR to AMP is more specific than BHR to MCH for both confirming a diagnosis of asthma and aiding the management of asthma[2,9,10]. Although BCTs are increasingly recognized useful in the diagnosis and management of patients with asthma, their use may be limited by practical considerations, particularly in the pediatric population[3].

Exhaled nitric oxide (eNO) has been used increasingly as a noninvasive biomarker of airway inflammation in patients with asthma because it is simple, precise, reproducible, and provides immediate results[11,12]. Markers of airway inflammation, such as eosinophils from induced sputum^[13] and airway biopsy specimens^[14] have been found to correlate with eNO^[15] or BHR^[4] in patients with steroid-naïve asthma. Moreover, the results of recent studies-that included healthy and asthmatic patients from both pediatric and adult populations-indicated a strong relationship between eNO and atopy and degree of atopy as well[16,17]. However, few studies evaluated whether a relationship between BHR, eNO, and atopy exists in asthmatic patients and the results of these studies have been inconsistent[18-23]. Unless the interrelationship between BHR, airway inflammation, and atopy is clarified, utilization of these major components of asthma as objective measurement tools for confirming a diagnosis

of asthma will lack the rationale. Accordingly, it is important to document whether eNO may be used as a noninvasive surrogate marker that predicts BHR to AMP in patients with atopic or non-atopic asthma.

To our knowledge, no study that assesses eNO as a surrogate marker that predicts BHR to AMP in both atopic and non-atopic steroid-naïve children with asthma exists in the literature. The aim of this study is to document whether eNO may be used as a surrogate marker that predicts BHR to AMP in both atopic and non-atopic steroid-naïve school children with asthma.

METHODS

Patients

This is a retrospective cross-sectional analysis of the database of steroid-naïve asthmatic school age children who had been followed-up in our clinic with a diagnosis of asthma for at least two years. All patients had been challenged with MCH and AMP and eNO levels had been measured for inclusion in clinical trials. A definitive diagnosis of asthma was made according to Global Initiative for Asthma^[24] (GINA) guidelines, i.e., a history of recurrent cough, wheezing, and dyspnea on exposure to upper respiratory tract infections, irritants, and/or airborne allergens, together with reversibility of airflow limitation (an increase in forced expiratory volume in 1 s (FEV₁) $\geq$ 12% after inhaled short-acting β_2 -agonist administration) or favourable response to inhaled corticosteroids and/or inhaled bronchodilators. Exclusion criteria were as follows: patient younger than six years of age, patient with a history of any other chronic pulmonary diseases and of near-fatal asthma or major exacerbations necessitating the use of systemic corticosteroid, patient who received inhaled corticosteroid before BCTs and measuring eNO were excluded. Our study had been

reviewed and approved by the Gazi University Faculty of Medicine Ethic Committee and written informed consent was obtained from each patient and/or parents.

Skin-Prick Tests

Skin-prick tests (SPT) were performed according to the EAACI^[25] guidelines, using ten common aeroallergens: house dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), pollens (*grass mix*, *weed mix*, *cereal mix*, and *tree mix*), animal danders (*cat epithelia*, *dog epithelia*), alternaria, and cockroach (Stallergens, France). Atopy was defined as at least one skin prick test with a wheal diameter of 3 mm or greater than the negative control at 15 min after administration. In addition to the number of positive SPTs, the wheal size of positive SPTs was assessed and wheal diameters were graded as follows: a wheal diameter of 3.0-5.4 mm was scored 1, a diameter between 5.5-8.4 mm was scored 2, 8.5-11.4 mm scored 3, and ≥ 11.5 mm scored 4. Finally, the scores were summed up to give an atopy score for each patient[8]. Total serum IgE levels were determined using a chemiluminescence automatic immunoassay (Beckman Coulter, Fullerton, CA, USA) and expressed in IU/ml.

Bronchial Challenge Tests

Methacholine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and AMP (Sigma-Aldrich Chemie GmbH) were prepared daily from a stock solution in doubling concentrations from 0.031 to 16 mg/ml and 0.39 mg/ml to 400 mg/ml, respectively. They were delivered by a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA, USA) attached to a compressor which produced an output of 0.13 ml/sec (Pulmo aide compressor by DeVilbiss Co.,USA).

Each BCT was performed by 2-min tidal breathing method through a mouthpiece[26,27]. They were performed at the same time of day with an interval of at least 3 days. Pulmonary functions tests (PFTs) were measured according to American Thoracic Society^[28] (ATS) guidelines with a daily-calibrated spirometer (Vmax 20C, SensorMedics Co., Yorba Linda, CA, USA). Both BCT procedures were done as in our previous reports[29,30]. At the time of testing, all patients had been free of acute respiratory infection for a period of 6 weeks. Inhaled short- and long-acting β_2 -agonist were withheld for at least 8-24h. Antihistamines and the others medications were stopped 7 days before each challenge test, as well.

Exhaled Nitric Oxide

Exhaled NO was measured with a fast response chemiluminescence analyzer (NOA Tm 280i; Sievers Instruments Inc., Boulder, CO) by the on-line single breath technique, according to the ATS and European Respiratory Society (ERS) recommendations for children[31]. Measurements of eNO were always done before performing BCTs. The details of eNO measurement was written in detail in our previous report[30].

Statistical Analysis

After entering the data using Statistical Package for the Social Sciences (SPSS) Version 15.0 software (SPSS Inc., Chicago, IL, USA), the analysis of the results were performed using percentage distribution for qualitative data, the median (interquartile range (IQR)) or geometric mean (95% confidence interval (95%CI)) for quantitative data. The statistical tests used were the Shapiro-Wilks' test for normality, the chi square test or the Fischer's exact test for qualitative data comparison of groups, and the independent samples tests or the Mann - Whitney U for quantitative data comparison of groups, as appropriate. PC₂₀ AMP and PC₂₀

MCH values had to be converted logarithmically to normalize data. The correlation between logarithmically transformed (PC₂₀ AMP and PC₂₀ MCH) data and other quantitative data was calculated by Pearson's correlation test. Multivariate logistic regression analysis were used to evaluate identify independent predictive factors for BHR to AMP in both the atopic and the non-atopic groups. Family history of asthma, allergic rhinitis, eNO, PC₂₀ MCH, parameters of both atopy and PFTs, were included in the regression model with adjustment for age and sex. To compare the maximum efficiency, sensitivity, specificity, and positive (PPV) and negative predictive values (NPV) of eNO for the prediction of BHR to AMP, receiver operation characteristic (ROC) curves were constructed and analyzed in both the atopic and the non-atopic groups, in the determination of optimal cut off point by Youden J. The area under curve (AUC) and 95% CI were calculated by using MedCalc® (MedCalc Software, Mariakerke, Belgium) for both groups. A p value less than 0.05 was considered statistically significant.

RESULTS

One-hundred and sixteen patients (73 male) were included in the analysis. Demographic, clinic, and functional data were presented in Table 1. One-hundred-ten (94.8%) and sixty-one (52.6%) patients had BHR to MCH and BHR to AMP, respectively. The geometric mean (95% CI) of PC₂₀ MCH and of PC₂₀ AMP were 1.12 (0.37-7.29) mg/dl and 78.84 (0.39-800) mg/dl, respectively (Table 1). Three patients had a PC₂₀ AMP concentration between 201-400 mg/dl, they were defined as did not respond to AMP. Sixty-nine (59.5%) patients had atopy and they had significantly higher eNO levels compared to patients without atopy (44.53±19.1 ppb vs 25.62±12.9 ppb, respectively; p<0.001).

Table 2 demonstrates the correlations between eNO, PC₂₀ MCH, PC₂₀ AMP, PFTs parameters (FEV₁, FEV₁/FVC, and FEF₂₅₋₇₅), and atopy parameters (number of positive SPT, atopic score, serum total IgE level). The Spearman's correlation test revealed a significant relationship between PC₂₀ AMP and eNO (rho:-0.466, p<0.001), and between PC₂₀ AMP and PC₂₀ MCH (rho:-0.386, p<0.001) but not between eNO and PC₂₀ MCH (rho:-0.115, p=0.220) (Table 2). Both eNO and PC₂₀ AMP correlated significantly with the atopy parameters except for serum total IgE (Table 2). However, PC₂₀ MCH did not correlate significantly with any of the atopy parameters (p>0.05 for all of the parameters). Only the PFT parameters correlated significantly with PC₂₀ MCH (Table 2).

Comparison of groups according to presence of both atopy and of BHR to AMP

Demographic factors, atopy parameters, pulmonary function test results and geometric mean PC₂₀ MCH did not differ significantly between patients with BHR to AMP and without BHR to AMP in neither the atopic nor the non-atopic groups (p>0.05 in all of the parameters) (Table 1).

The number of patients with BHR to AMP was significantly higher in the atopic group compared to patients with BHR to AMP in the non-atopic group (p=0.013). The geometric mean PC₂₀ AMP was significantly lower in patients with BHR to AMP in the atopic group compared to the non-atopic patients with BHR to AMP (p=0.003). The highest mean eNO level was observed in patients with BHR to AMP in the atopic group (Figure 1). In the atopic group, eNO values were significantly higher in patients with BHR to AMP compared to those without BHR to AMP (51.9±16.9 ppb vs 33.7±16.4 ppb; p<0.001), whereas in the non-atopic group the differences were not statistically significant (29.7±16.9 ppb vs 22.6±8.1 ppb;

p=0.152) (Figure 1) (Table 1). When multiple logistic regression analysis was performed, eNO levels (R^2 :0.401; β :0.092; 95%CI: 1.19-14.42; OR: 7.12; p=0.008) were found to be the only independent factor for BHR to AMP in the atopic group, whereas none of the parameters predicted BHR to AMP in the non-atopic group.

ROC analysis

ROC analysis was performed, in both the atopic and the non-atopic groups, to evaluate the ability of eNO to detect the BHR to AMP (Figure 2). The best cut-off value of eNO that significantly predicts BHR to AMP was 33.3 ppb in the atopic group (p<0.001) and sensitivity, specificity, PPV, and NPV of eNO value was 78.36%, 74.47%, 81.8%, 70.15%, respectively (Figure 2A) (Table 3). The best cut-off value for eNO that significantly predicts BHR to AMP in the non-atopic group could not be documented (p=0.142) (Figure 2B). The sensitivities, specificities, positive likelihood ratio (+LR), negative likelihood ratio (-LR), PPV and NPV were calculated and presented at selected decision points of the ROC curve in the atopic group (Table 3). An $eNO \leq 17.4$ ppb has 100% NPV and 100% sensitivity and 60.47% PPV for prediction of BHR to AMP in the atopic group.

DISCUSSION

To our knowledge, this is the first study that designates eNO as a surrogate marker which predicts BHR to AMP in school age children with steroid-naïve asthma. A significant cut-off value for eNO to predict BHR to AMP was found only in patients with atopic asthma but not in non-atopic asthma. An eNO value of 33.6 ppb was found to predict BHR to AMP with a 78.36% sensitivity and a 74.47% specificity. It was shown that eNO less than 17.4 ppb had a

NPV of % 100 for unresponsiveness to AMP. However, a similar relationship was not found between eNO and BHR to MCH.

Until recently, little had been published regarding the efficacy of eNO as a surrogate marker predicting BHR in asthmatic patients and all studies- except one- included asthmatic adults[18-23]. In three studies eNO was not found a reliable tool to predict BHR to direct stimuli such as MCH or histamine[18-21]. We also did not find a correlation between BHR to MCH and eNO. There is one study that investigated the relationship between eNO and both direct and indirect stimuli to BHR[22]. In that study, Berkman et al.^[22], showed that eNO > 7 ppb may be used as a surrogate marker to predict BHR to AMP, exercise and MCH for diagnosis of asthma in adults with suspected symptoms. In their study Berkman et al.^[22] defined a cut off value of 7 ppb with a flow rate of 250 ml/s. In the present study, we defined a cut off value of 33.6 ppb with a flow rate of 50 ml/s. Therefore, the difference between these cut off values can be attributed to using dissimilar flow rates of eNO. In another study, the authors used exercise as an indirect stimulus, and documented that eNO could predict BHR to exercise challenge test in children with uncontrolled asthma[23]. Our results overlap with them with respect to the relation between eNO and indirect stimuli but not with direct stimuli. We think that this is due to the complex pathophysiologic mechanisms involved in BHR and its complex relationship with airway inflammation[1,2].

Although there is a relationship between eNO and AMP, both parameters were well known to be affected by atopic sensitization and anti-inflammatory treatment[6-8,11,14,17]. Therefore, we compared this relationship between eNO and AMP in atopic and nonatopic steroid-naïve children with asthma. Our findings showed that eNO was the only independent variable for

BHR to AMP in the atopic group. The AUC, sensitivity, PPV and NPV of eNO was significant in the atopic group. Moreover, in the atopic group eNO was significantly higher in patients with BHR to AMP compared to patients without BHR to AMP. Moreover, eNO <17.4 ppb had a NPV of 100% for prediction of BHR to AMP. Similarly, Buchvald et al.^[23] evaluated exercise-induced bronchoconstriction and reported that eNO < 20 ppb had a NPV of 100% for prediction of BHR to exercise-challenge test. Another study demonstrated that eNO <20 ppb had a NPV of 92% among patients with suggestive symptoms of asthma[32]. According to recent ATS guideline recommendations an eNO value more than 20 ppb was suggestive of asthma in children[11]. Finally, it can be stated that a cut-off eNO value of < 17.4 ppb is an objective, reliable, and non-invasive tool for the exclusion of asthma in atopic children. Exhaled NO may have potential to replace AMP bronchial challenge test for the evaluation of BHR.

In the present study, mean FEV₁, FEV₁/FVC, and FEF₂₅₋₇₅ values were normal. This may be attributed to the fact that almost all of participants of the study had mild asthma. To evaluate reversible airway limitation PFTs, in combination with clinical findings, have been used commonly as an objective diagnostic tool for the diagnosis of asthma[24]. However, reversibility of airway limitation and/or airway limitation alone may not be observed in patients with mild and well-controlled asthma[24, 32]. It is documented that PFT, including testing for reversibility of airway limitation, has a limited sensitivity for diagnosing asthma of approximately 25% depending on the patient population examined[33]. In addition, a gold standard test does not exist for diagnosis of asthma and all patients do not present with an unambiguous clinical picture[32]. These facts may lead to both under- and over diagnosis of asthma[34]. Among patients with symptoms suggestive of asthma who present with normal pulmonary function, an objective measurement such as a BCTs and/or markers of airway

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inflammation may help establish a diagnosis of asthma[3,24,32]. There are more accurate methods for determining airway inflammation such as bronchoalveolar lavage and/or transbronchial biopsy. However, they are invasive and may lead to serious complications which renders these methods impractical for clinical use[15]. Exhaled NO has been used increasingly as a noninvasive biomarker of airway inflammation in both diagnosis and management of patients with asthma[11]. Diagnostic efficacy of eNO could not be defined clearly yet because of two reasons: there is a lack of a gold standard diagnostic test for asthma diagnosis and some practical factors related to eNO measurement[35]. Furthermore, BCTs that are utilized in the determination of BHR have similar limitations[2]. The major difference between direct and indirect BCTs, in general practice and results of previous clinical studies, is the sensitivity and specificity of each BCT in diagnosing asthma[2]. In contrast to BCT with direct stimuli such as MCH, BCT with indirect stimuli such as AMP, mannitol and exercise are more specific and thus more valuable for confirming a diagnosis of asthma and to evaluate treatments with anti-inflammatory drugs[3,10]. However, indirect stimuli entail the presence of airway inflammation[2]. Thus, evaluation of response to these stimuli identifies the interaction of the two key features of asthma, airway inflammation, and BHR. Recent studies conducted by us^[7] and other centers^[4,6,8] revealed that frequency of BHR to AMP (46%, 90%, 88.5%, and 89%, respectively) was in close harmony with frequency of atopy (%33, 78%, 79%, and 100%, respectively). Moreover, Suh et al.^[6] reported a strong relationship between BHR to AMP and degree of atopy. Similarly, a strong relationship between eNO and atopy has been reported[16,17]. Our results demonstrate that when an objective marker is needed for the diagnosis of asthmatic children with atopy, eNO (non-invasive, simple and yielding immediate results) may be used instead of other BCTs (time-consuming, necessitating experienced staff and special equipment, affecting patient physiology).

Mannitol is the indirect stimulus of choice that is utilized to determine BHR due to the fact that it has a validated and standardized application protocol that is simple and workable[3,10]. Both eosinophilic and non-eosinophilic phenotypes have been reported in asthma patients responsive to BCT with mannitol[3]. Although a proportion of asthmatics who have BHR to mannitol have eNO values well within the normal range, there are studies identifying significant relationship with eNO-a non specific marker of airway inflammation- with the BHR to mannitol[36-38]. Moreover, the relationship between atopy and BHR to mannitol is not well-documented compared to the relationship between atopy and BHR to AMP which is documented in detail[3,6-8]. Additionally, studies comparing BCT with mannitol and BCT with AMP is scarce in the literature[3,38,39]. Therefore, further research comparing the relationship between indirect stimulants of BCTs (AMP and mannitol) and evaluating the effect of atopy on this relationship is crucial.

In the non-atopic group, eNO was not a significant surrogate marker for predicting BHR to AMP. Although statistically insignificant, eNO levels were higher in non-atopic patients with BHR to AMP, compared to patients without BHR to AMP. It may be due to mechanisms of NO production which may differ in patients with atopic and non-atopic asthma yet how they differ remains unclear[11,17,40]. There is accumulating evidence that NO and NO metabolites in the airway (e.g. peroxynitrite) alter the REDOX balance in the airway thus bronchial NO production is, to some extent, independent of eosinophilic inflammation[40]. On the other hand, non-eosinophilic airway inflammation may be a result of high eNO levels. It is previously reported that eNO correlates strongly with both the degree of atopy and the presence of atopy[16,41,42]. However, in an adult study, Langley et al. documented that high exposure to house dust mite and dog was associated with worse BHR and elevated eNO even in the absence of atopy[43]. Moreover, a community-based study has shown that subjects

exposed to high levels of cat allergen, even when not sensitised to cat, have worse BHR and elevated eNO[44]. To conclude, it is clear that allergens have non-IgE mediated effects on eNO[45]. Previous studies have shown controversial results concerning interrelationship between asthma, atopy and eNO[46]. Recent research reported high eNO mainly in atopic children independent of asthma status, while others have found higher eNO levels in atopic children with concomitant asthma, compared to those with atopy alone[15,42,47].

Sensitivity, specificity, PPV, and NPV of eNO was moderate even with the best cut off values as a surrogate marker in predicting BHR to AMP in the atopic group. It may be due to the currently unknown factors effecting eNO levels[11]. This finding may also be related to the factors other than airway inflammation that affect BHR. Degree or intensity of BHR may be one of these factors which may vary considerably among patients with asthma and within individuals themselves, as well[1]. However, there is no known classification of severity of BHR to AMP although it is well known for BHR to MCH[26,28]. Variability of degree or intensity of BHR may be related to the severity of the underlying asthma, variability of airway caliber, intercurrent provokers, such as allergen exposure, viral-induced exacerbations, non-specific airway irritation or the patients' anti-inflammatory treatment[48-50]. In our study, none of the children had a history of respiratory infection six weeks before the study period and none received antiinflammatory treatment before the diagnosis of asthma was established. In addition, almost all of the participants of the study were diagnosed as mild persistent asthma. However, we could not measure exposure to allergens and non-specific airway irritants, variability of airway caliber and remodeling which may limit our results. Another limitation of our study is its retrospective design.

To conclude, our findings suggest a strong relation between atopy and BHR to AMP and eNO in steroid naïve children with asthma. Exhaled NO may be used to predict BHR to AMP in

atopic but not in non-atopic steroid naïve asthmatic children. However, prospective studies are needed to verify our results.

Conflict of Interest Statement and Financial Disclosure

The authors have no conflicts of interest relevant to this study. There was no financial support for this study. All authors contributed significantly to the present study, they have approved and take full responsibility for the final manuscript.

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Figure Legends

Figure 1 Exhaled NO levels between groups according to presence of both atopy and BHR to AMP

Figure 2 ROC curve for ability of eNO to predict BHR to AMP. (A) in atopic group, (B) in non-atopic group.

Table 1 Demographic, clinical, and functional data in the all, the atopic and the non-atopic groups according to the presence of BHR to AMP

	The atopic group (n=69)		The non-atopic group (n=47)		The all group (n=116)
	Responsive to AMP (n=41)	Unresponsive to AMP (n=28)	Responsive to AMP (n=20)	Unresponsive to AMP (n=27)	
Demographic characteristic					
Age, years	11.97±2.6	12.35±2.2	11.6±2.6	11.4±2.6	11.8±2.4 (7-16)
Sex (M/F), ratio	28/13	17/11	13/7	15/12	73/43
Family history of asthma, n (%)	16 (39.0)	15 (53.6)	9 (45.0)	11 (40.7)	51 (44.0)
Allergic rhinitis, n (%)	25 (60.9)	19 (67.8)	-	-	44 (37.9)
Atopic eczema, n (%)	1 (2.4)	1 (3.6)	1 (5.0)	1 (3.7)	4 (3.4)
Obesity, n (%)	4 (9.6)	2 (7.2)	3 (15.0)	3 (11.1)	12 (10.3)
PFT parameters					
FEV ₁	0.86 (0.66-1.12)	1.22 (0.73-1.93)	0.98 (0.74-1.41)	1.33 (0.89-2.1)	1.12 (0.37-7.29)
FEV ₁ /FVC	7.38 (4.37-12.4)*	718.2 (633-800)	20.12 (8.69-46.6)	800 (800-800)	78.9±22.1 (72-123)
FEF ₂₅₋₇₅	51.9±16.9**	33.7±16.4	29.7±16.9	22.6±8.1	1.12 (0.37-7.29)
PC ₂₀ MCH, mg/ml	4.2±2.3	3.5±1.7	-	-	78.84 (0.39-800)
PC ₂₀ AMP, mg/ml	5.4±3.3	4.3±2.6	-	-	38.86±19.2 (5.2-87.0)
eNO, ppb	346.1±275.3	286.3±407.5	209.1±285.1	52.5±55.1	
Atopy parameters					
Number of positive SPT					2.67±2.6 (0-8)
Atopy score					3.33±3.5 (0-12)
Serum total IgE level, IU/ml					221.1±301.5 (18-1819)

Abbreviations: BHR, bronchial hyperresponsiveness; PFT; pulmonary function test; AMP, adenosine monophosphate; PC₂₀, provocative concentration of MCH causing 20% decreased in FEV₁; eNO; exhaled nitric oxide; SPT, skin prick test.

Data with¹ or without² a normal distribution were presented as mean (SD) or geometric mean (95% CI), respectively.

[‡]Pre-test baseline values (% predicted)

*p<0.05 comparison of response to AMP in the atopic and the non-atopic groups, **p<0.001 compared with between atopic groups.

Table 2 Correlations between BHR (PC₂₀ AMP and PC₂₀ MCH), eNO, and parameters of atopy and PFT in all patients

	eNO		PC ₂₀ AMP		PC ₂₀ MCH	
	ρ	p	ρ	p	ρ	p
BHR						
PC ₂₀ AMP	-0.466	<0.001				
PC ₂₀ MCH	-0.115	0.220	0.386	<0.001		
Atopy parameters						
Number of positive SPT	0.444	<0.001	-0.262	0.004	-0.065	0.489
Atopy score	0.460	<0.001	-0.277	0.003	-0.075	0.425
Serum total IgE level	0.133	0.230	-0.123	0.268	-0.035	0.570
PFT parameters						
FEV ₁	0.031	0.740	0.144	0.122	0.222	0.017
FEV ₁ /FVC	0.009	0.926	0.101	0.279	0.187	0.044
FEF ₂₅₋₇₅	0.080	0.396	0.184	0.184	0.195	0.036

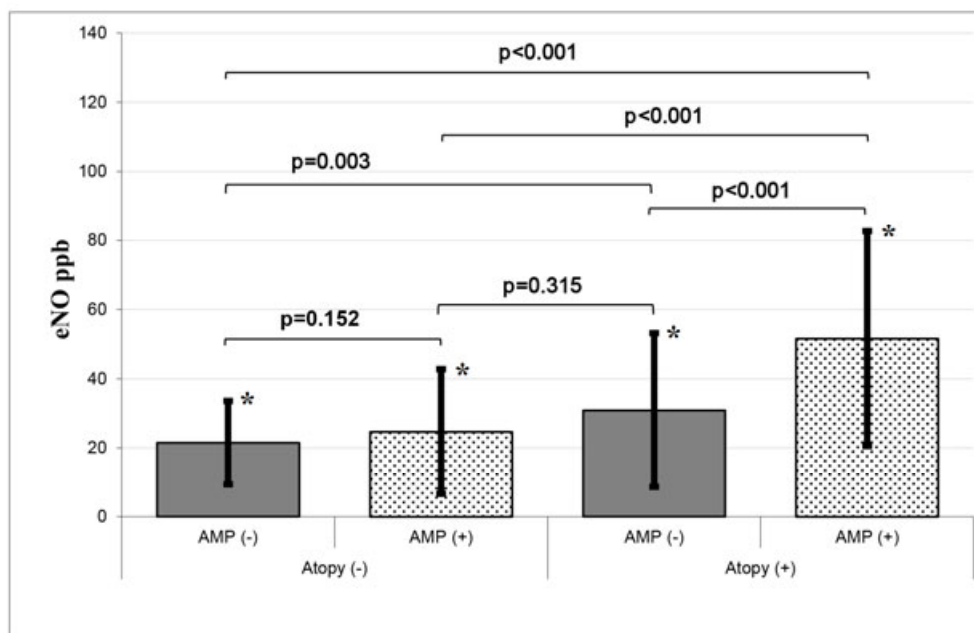
Abbreviations: BHR, bronchial hyperresponsiveness; AMP, adenosine monophosphate; PFT, pulmonary function parameters; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF₂₅₋₇₅, forced expiratory flow at 25-75% of maximal lung volume; PC₂₀, provocative concentration of AMP or MCH causing 20% decreased in FEV₁; eNO; exhaled nitric oxide; SPT, skin prick test; ρ : Spearman's rho.

Table 3 Results of the selected cut-off levels of eNO that predicts BHR to AMP in the atopic group

	Cut-off point for eNO (ppb)	Sensitivity % (%95 CI)	Specificity % (%95 CI)	+LR (%95 CI)	-LR (%95 CI)	PPV	NPV
The Atopic group	17.4	100.0 (94.8-100)	4.26 (0.5-14.5)	1.04 (1.0-1.1)	-	60.47	100.0
	20.0	98.55 (92.2-100)	14.89 (6.2-28.3)	1.1	0.09	62.89	87.39
	25.0	92.75 (83.9-97.6)	38.30 (24.5-53.6)	1.16 (1.0-1.3)	(0.01-0.8)	68.76	78.30
	30.0	97.6	53.6	1.3	0.18	76.14	72.56
	33.3*	84.16 (69.9-89.6)	61.40 (46.4-75.5)	1.51 (1.2-2.1)	(0.06-0.5)	81.81	70.15
	40.0	89.6	75.5	2.1	0.25 (0.1-0.5)	81.39	60.53
	45.0	78.36 (63.5-84.9)	74.47 (59.7-86.1)	2.18 (1.4-3.1)	0.29 (0.2-0.5)	83.31	57.41
	50.0	84.9	86.1	3.1	0.29 (0.2-0.5)	88.09	56.83
	55.0	65.22 (52.8-76.3)	78.20 (64.3-89.3)	3.06 (1.6-5.4)	0.44 (0.3-0.6)	90.01	51.23
		57.97 (45.5-69.8)	82.98 (69.2-92.4)	3.11 (1.7-5.5)	0.50 (0.4-0.7)		
		53.62 (41.2-65.7)	89.36 (76.9-96.5)	3.41 (1.8-6.6)	0.51 (0.4-0.7)		
		39.13 (27.6-51.6)	93.62 (82.5-98.7)	5.03 (2.1-11.9)	0.65 (0.6-0.8)		
				6.13 (2.0-19.0)			

Abbreviations: eNO, exhaled nitric oxide; NPV, negative predictive value; PPV, positive predictive value; +LR, Positive Likelihood Ratio; -LR, Negative Likelihood Ratio;

*Best cutoff point for the atopic group: AUC=0.801 (95%CI: 0.687-0.887); Z=5.762; p<0.001



*The values are presented as mean±2SD

