Narongwit Nakwan, Thidarat Ruklerd, Thitima Perkleang, Pattarawadee Taptawee

Hatyai Medical Education Center, Hatyai Hospital, Songkhla, Thailand

The levels and correlations of FeNO, blood eosinophils and lung function in well-controlled asthma

Abstract

Introduction: Whether biomarkers (i.e., fractional exhaled nitric oxide (FeNO) and blood eosinophils) or lung function are additional ultimate outcomes in asthma treatment among patients with clinical remission has been the subject of previous research, the study of the correlations between FeNO, blood eosinophils and lung function among well-controlled asthmatic patients is less clear. To investigate the clinical application of the correlation between FeNO, blood eosinophils and lung function parameters in well-controlled asthmatic patients.

Material and methods: This was a prospective cross-sectional study. We measured FeNO, blood eosinophil and lung function in 84 asthmatic patients with clinical remission who were assessed by asthma control questionnaires. The correlation coefficient was used to ascertain among those parameters. The diagnostic accuracy of blood eosinophil to identify low FeNO (< 25 ppb) was calculated using the area under the receiver operating characteristics (AUROC).

Results: Of 84 patients analyzed, the median ACT was 25 and the median ACQ-7 was 0.43. The median duration of being well-controlled asthma was 14.5 months. The median FeNo was 23 ppb and the median blood eosinophils was 375 cell/mm^3. A significant positive correlation was found between FeNo and blood eosinophil ($r = 0.310$, $p = 0.004$). No correlation was detected between either FeNO or blood eosinophil and all lung function parameters. The AUROC results for blood eosinophils was 64.4% ($p = 0.024$) to detect FeNO < 25 ppb at a cutoff point of 295 cell/mm^3 (sensitivity = 83.5%, specificity = 50%).

Conclusions: Measuring FeNO and blood eosinophils in patients with a clinical remission of asthma may determine which of those patients have achieved complete remission. As the level of blood eosinophils has a significant correlation with FeNO, it may easily be a feasible biomarker to evaluate inactive airway inflammation before stepping down asthma treatment.

Key words: eosinophil, fractional exhaled nitric oxide, lung function, asthma, biomarker

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Introduction

Asthma is a heterogeneous disease of the airways that still remains a highly prevalent condition and one which is associated with extensive exacerbations, health care utilization and expenditure [1]. According to the guidelines of the Global Initiative for Asthma (GINA), the ultimate goals of treatment are the achievement of symptom control, the maintenance of normal activity levels, and the minimization of future risk of exacerbations and fixed airflow limitations [2]. However, stepping down treatment is also recommended when patients have achieved clinical remission of asthma with optimal lung function [2]. This strategy could minimize the costs of treatment and the potential for side-effects [3]. Although all aspects of the disease control, including reaching asthma control, suppressing airway inflammation, stabilizing lung function, and preventing airway remodeling, are the major goals of asthma management [4], GINA routinely recommends stepwise treatment based only on controlling the symptoms without monitoring for airway inflammation. Although testing biomarkers could determine the inflammatory phenotype of patients and help clinicians to optimize therapy decisions [5], the study of

Address for correspondence: Narongwit Nakwan, Hatyai Medical Education Center, Hatyai Hospital, 182, 90110 Songkhla, Thailand, e-mail: naronak@hotmail.com

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biomarkers in patients with clinical remission of asthma during the stepping down period is still lacking. Only one previous study found that sputum eosinophilia could be a predictive marker for a loss of asthma control during dose reduction of inhaled corticosteroids [6], but there was not enough data in that clinical research to study the biomarkers. Sputum eosinophilia is primarily a key inflammatory marker to determine circulating Th2 cytokines and eosinophilic airway inflammation [7], and also to predict favorable asthma outcomes [8], but this biomarker is more difficult to establish due to the complex technique involved and the fact that it is not generally available in clinical settings. Therefore, blood eosinophil and fractional exhaled nitric oxide (FeNO) have become alternative biomarkers to determine a strong association with eosinophilic inflammation [9, 10] due to their having a good correlation with sputum eosinophilia [11, 12]. In addition, blood eosinophilia increases the risk of future exacerbations, the development of fixed airway obstruction, readmission [13, 14], and the use of health care resources [15], while FeNO is also associated with asthma control and future risk of exacerbation, meaning that it could help clinicians to adjust the appropriate dose of inhaled corticosteroids [12, 16–18]. Both measurements of FeNO and blood eosinophils are non-invasive, simple, and safe methods of measuring airway inflammation which can be performed in routine clinical practice. Several studies have tried to establish a correlation between blood eosinophils and FeNO, but the data are still inconclusive. One previous study found no correlation between FeNO and blood eosinophil percentages/counts even in uncontrolled asthma [19], whereas another study showed that FeNO was significantly correlated with blood eosinophil levels in uncontrolled childhood asthma [20].

Although the assessments of asthma control and lung function are simple tools to guide clinicians in stepping down those asthma medications, the clinical application of the levels and correlations of FeNO, blood eosinophils and lung function in patients with clinical remission of asthma is controversial. This investigator-initiated study was designed to describe the levels of FeNO, blood eosinophils and lung function parameters in patients with well-controlled asthma and assess the correlations between FeNO, blood eosinophils and lung function parameters in these patients.

Material and methods

Patients

The cohort enrolled into this study was patients with well-controlled asthma who visited an asthma clinic in Hatyai Regional Hospital, Songkhla, Thailand. They had previously been diagnosed with asthma by performing spirometry in accordance with the standards of the American Thoracic Society [21]. The inclusion criteria were as follows: being over 18 years old; having been treated with step-3 management based on the 2020 GINA guidelines (only low dose inhaled corticosteroid/long acting beta agonist (ICS/LABA) maintenance) for at least 6 months [2]; meeting the well-controlled criteria of an Asthma Control Test (ACT) score ≥ 23 and an Asthma Control Questionnaire 7-item version (ACQ-7) score ≤ 0.75; and being able to perform spirometry at date of enrollment into the study. Patients were excluded if they were current smokers or had a smoking history of >10 pack-years, and/or had other chronic pulmonary disease such as chronic obstructive pulmonary disease, chronic bronchitis, lung cancer, previously infected pulmonary tuberculosis or pulmonary fibrosis. Patients who had a history of previous lung infection and asthma exacerbation within 12 weeks and had been taking systemic corticosteroid within 12 weeks were also excluded.

Study design and treatments

This was a prospective cross-sectional study, single center trial (ClinicalTrials.gov identifier NCT04454385) conducted from August 2020 to October 2020. The study was performed in accordance with the principles of the Declaration of Helsinki, and was consistent with the International Conference on Harmonization and Good Clinical Practice and applicable regulatory requirements. The protocol was approved by the institutional review board, and informed written consent was obtained from each participant prior to their participation in the study. The patients were advised that all necessary measurements would be taken in one hospital visit. During this visit, inclusion and exclusion were evaluated, and a pulmonary function test and bronchodilator reversibility test were performed. Inflammatory status was measured by FeNO and blood eosinophils. Baseline characteristics including age, sex, height, body weight, body mass index, smoking history, current medications, comorbidity, and duration of diagnosed asthma were collected from

face-to-face interviews with the patients and from their their medical records.

Measurements

Lung function test

Lung function tests was performed using a spirometer, VIASYS®, (CareFusion, California, USA) according to the standards of the American Thoracic Society [21]. The highest of three values of pre-dose forced expiratory volume in 1 second (FEV1), repeatable within 5%, was recorded and the predicted percent was calculated. The percentage predicted values (% pred) were calculated based on reference values for healthy Thai adults. Forced vital capacity (FVC), pre-dose FEV_1, and the ratio of FEV_1/FVC were evaluated, and the peak expiratory flow rate (PEFR) and mid maximal expiratory flow (MMEF) were collected. Bronchodilator reversibility tests were performed by inhaling 400 μg salbutamol via metered-dose inhaler after baseline testing. The percentage of reversibility was calculated and collected based on changes in the FEV_1 or FVC before and after salbutamol inhalation.

Fractional exhaled nitric oxide

The FeNO level was measured by using a portable device, NObreath®, (Bedfont Scientific, UK) that measures the level of nitric oxide in parts per billion (ppb) in the air. The patients were asked to refrain from eating nitrate rich food, drinking caffeine and alcohol, and smoking for a least 2 hours before the test. The patients exhaled slowly with an expiratory air flow of 50 mL/sec from their total lung capacity. The mean value of two correctly performed measures was used for analysis. The FeNO levels were classified as recommended by the American Thoracic Society for adults [12]. A FeNO level of less than 25 ppb indicates those well controlled asthmatic patients were receiving adequate dosages of medication and achieving good adherence to their anti-inflammatory therapy [12]. In this study, the level of FeNO was categorized into two groups: the first one was defined as high FeNO (FeNO ≥ 25 ppb) and the second was defined as low FeNO (FeNO < 25 ppb).

Blood eosinophils

Peripheral blood eosinophil counts were obtained from standard complete blood count analysis. The absolute count and percentage of eosinophils were collected. We selected a threshold of ≥ 400 cell/mm^3 which showed an association with worse clinical asthma outcomes as reported in researches in the literature [15–24]. In this study, the level of blood eosinophils was categorized into two groups: the first one was defined as high blood eosinophil (≥ 400 cell/mm^3) and the second was defined as low blood eosinophil (< 400 cell/mm^3).

Asthma control test

The ACT was comprised of four symptom/reliever questions plus a patient self-assessed level of control on the following five indicators in the preceding 4 weeks: limitation of activities; shortness of breath; awakenings at night; use of reliever medication; and patient's perception of asthma control. Each question had five response options, resulting in a score range of 1–5. The total score, therefore, ranged from 5–25 (with a higher score indicating more controlled asthma). A score of 20–25 is classified as well-controlled asthma; 16–20 as not well-controlled; and 5–15 as very poorly controlled asthma [25, 26].

Asthma control questionnaire score

ACQ-7 is the questionnaire that was used in this study to measure the adequacy of asthma control and changes in asthma control which occurred either spontaneously or as a result of treatment. ACQ-7 has a multidimensional construct assessing symptoms (5 items self-administered), rescue bronchodilator use (1 item self-administered), and FEV_1% (1 item completed by clinic staff). The total score ranged from 0–6 (with a higher score indicating worse asthma control). A score of 0.0–0.75 is classified as well-controlled asthma; 0.75–1.5 as a 'grey zone'; and > 1.5 as poorly controlled asthma [27–29].

Statistical analysis

All statistical analyses were conducted using SPSS Statistics version 23 for Windows. The patient demographic data were summarized in frequency tables. Categorical values, expressed as numbers with proportions, were analyzed using Chi square test. Standard distribution was tested by the Skewness-Kurtosis technique. Continuous or ordinal values were summarized as either mean ± SD or median with an interquartile range. Standard distribution variables were analyzed by non-parametric Manne-Whitney U test. The relationship between FeNO, blood eosinophils, lung function parameters and bronchodilator reversibility were calculated using the Pearson's correlation coefficient for standard distribution variables and Spearman's rank correlation coefficient for non-standard distribution variables. To detect a correlation coefficient (r = 0.3) by using

a two-sided test, 5% significance level test ($\alpha = 0.05$) with 80% power ($\beta = 0.2$), the required sample size was 84. Statistical significance was defined as p-values < 0.05. The receiver-operating characteristic (ROC) curve determined the optimal cutoff value of blood eosinophils which best identified FeNO < 25 ppb from the highest sum of sensitivity and specificity.

Results

Baseline characteristics of the patients

A total of 84 patients with well-controlled asthma met the eligibility criteria in this study. The baseline characteristics of the patients are summarized in Table 1. Overall, the mean age was 55 ± 12 years, and 73.8% of the patients were female. In addition, 81% of the patients were non-smokers, while the median duration of asthma onset was 10 years with a range of between 5 and 30 years. The most common comorbidity diseases were allergic rhinitis (67.9%), gastroesophageal reflux (27.4%), sleep apnea (4.8%) and psychiatric problems (4.8%). The median duration of having well-controlled asthma status during step-3 management with low dose ICS/LABA was 14.5 months with a range of between 8.7 and 20.9 months. The results of the lung function tests are presented on Table 2. The patients with well-controlled asthma had a median FEV_1/FVC ratio of 71% with a range of between 63 and 78%, mean FEV_1% pred of 77.6 ± 15.3%, mean FVC% pred of 90.4 ± 14.7%, mean PEFR% pred of 93.3 ± 22.3% and a median percentage of bronchodilator reversibility of 4.5% with a range of between 1.25 and 10%.

The biomarkers for determining airway inflammation are summarized in Table 3. The median FeNO was 23 ppb with a range of between 15 and 39.7 ppb. The absolute eosinophil count was 375 cell/mm^3 with a range of between 250 and 560 cell/mm^3 and the percentage of blood eosinophil was 5% with a range of between 3 and 7%. In addition to neutrophil, the absolute neutrophil count was 3,965 cell/mm^3 in a range of between 3,257 and 5,045 cell/mm^3 and the mean percentage of blood neutrophil was 55 ± 10.5%.

FeNO

The characteristics of patients classified by their level of FeNO are summarized in Table 4. A high FeNo level was present in 36 patients, of whom 63.8% were female. Patients in the two groups showed insignificant different results for all variables.

Table 1. Patient demographics and baseline characteristics

Patient characteristics	All participants (n = 84)
Age (years), mean (SD)	55 (12)
Sex, No. (%)	
Male	22 (26.2)
Female	62 (73.8)
Mean height, cm (SD)	156.9 (7.6)
Weight, kg (IQR)	61 (56.6, 70.9)
Body mass index, kg/m^2 (IQR)	25.6 (23.1, 28.6)
Smoking status No. (%)	
Former smoker	16 (19)
Non-smoker	68 (81)
ACT (IQR)	25 (24, 25)
ACQ-7 (IQR)	0.43 (0.14, 0.57)
Duration of diagnosed as asthma, years (IQR)	10 (5, 30)
Comorbidities (%)	
Allergic rhinitis	57 (67.9)
Gastroesophageal reflux	23 (27.4)
Psychiatric disease	4 (4.8)
Obstructive sleep apnea	4 (4.8)
Duration of status of well controlled asthma in step 3 GINA, months (IQR)	14.5 (8.7, 20.9)
Medication (%)	
Anti-histamine	39 (46.4)
Intranasal steroid	7 (8.3)

Values are shown as median (interquartile range), mean (SD) or number (%). ACT — asthma control test; ACQ-7 — asthma control questionnaire 7-item version; GINA — Global Initiative for Asthma; kg — kilogram; m — meter; No — number

Table 2. Spirometry data for all participants

Variables	All participants (n = 84)
FVC, L (IQR)	2.5 (1.9, 3.0)
FVC % predicted, mean (SD)	90.4 (14.7)
FEV_1 (L), mean (SD)	1.8 (0.6)
FEV_1 % predicted, mean (SD)	77.6 (15.3)
FEV_1/FVC (IQR)	0.71 (0.63, 0.78)
PEF (L/min), mean (SD)	5.7 (1.7)
PEF % predicted, mean (SD)	93.3 (22.3)
MMEF 25-75% (IQR)	1.2 (0.8, 1.8)
MMEF 25-75 % predicted (IQR)	46.5 (30, 65.5)
Bronchodilator reversibility, % (IQR)	4.5 (1.25, 10)

Values are shown as median (interquartile range) or mean (SD). FEV_1 — forced expiratory volume in 1 second; FVC — forced vital capacity; L — liter; LPM — liter per minute; MMEF — maximum mid-expiatory flow; PEF — peak expiratory flow

Blood eosinophils

High blood eosinophil levels were present in 54 patients. The characteristics of patients

Table 3. FeNo levels and peripheral blood results for all participants

Variables	All participants (n = 84)
FeNO level, ppb (IQR)	23 (15, 39.7)
White blood cell, cell/mm^3 (IQR)	7,020 (6,452, 8,797)
Eosinophil, % (IQR)	5 (3, 7)
Eosinophil count, cell/mm^3 (IQR)	375 (250, 560)
Neutrophil (%), mean (SD)	55 (10.5)
Neutrophil count, cell/mm^3 (IQR)	3,965 (3,257, 5,045)

Values are shown as median (interquartile range) or mean (SD). FeNO — fractional exhaled nitric oxide; mm — millimeter; ppb — parts per billion

classified by their level of blood eosinophil are summarized in Table 5. Patients in both groups showed insignificant different results for all variables except for a significant increase in the median FeNO to 27.5 in patients with high eosinophil levels compared with a median FeNO of 16.2 in patients with low eosinophil levels ($p = 0.003$).

Correlation between the FeNO level, blood eosinophil level and lung function test

The correlations between the FeNO levels, blood eosinophil levels and lung function parameters in the patients with well-controlled asthma are depicted in Figure 1. There was a significant positive correlation between FeNO and blood eosinophil levels ($r = 0.310$, $p = 0.004$), but the correlations between either FeNO or blood eosinophils and lung function parameters were insignificant (Figure 1). According to the lung function parameters, significant negative correlations were observed between bronchodilator reversibility and FEV1% predicted ($r = –0.574$, $p < 0.001$), PEFR% predicted ($r = –0.602$, $p < 0.001$), and MMEF% predicted ($r = –0.602$, $p < 0.001$).

The ROC curve analysis for blood eosinophil levels to detect FeNO < 25 ppb was 64.4% of the area under the curve ($p = 0.024$) (Figure 2). The optimal cutoff point for blood eosinophil levels was 295 cell/mm^3 and yielded 83.5% sensitivity and 50% specificity.

Discussion

The results of our study suggest that monitoring the levels of FeNO and blood eosinophils may indeed aid in determining the achievement of optimal complete remission of asthma and making a correct decision on when and how to step down medications in an individual during clinical remission of asthma merely assessed by the categorical scale using by ACT and ACQ. In this study, we found that the levels of both blood eosinophil and FeNO were low, and they had a significant relationship with each other. In addition, the level of blood eosinophils (< 295 cell/mm^3) was accurately able to predict those with low FeNO (< 25 ppb) with yielding sensitivity of 83.5% and specificity of 50% among the asthmatic patients experiencing clinical remission. However, lung function in the those patients with clinically well-controlled asthma appears to be independent of both biomarkers.

Exhaled nitric oxide is a highly reactive molecule and is recognized as playing key roles in the pathophysiology of lung disease, including asthma [30]. Generally, a high level of FeNO is expressed in asthma patients with active eosinophilic inflammation and is also represented as predisposing to the development of airway hyperresponsiveness (AHR) [31, 32]. Conversely, blood eosinophils are circulating cells that also become pivotal effector cells in inflammatory response to both allergic and non-allergic asthma reactions [33]. According to clinical implication, FeNO and blood eosinophils are established biomarkers of local and systemic active eosinophilic inflammation, respectively, and add a new dimension to the traditional tools for asthma management [9, 10]. Several studies have examined the ability of FeNO and blood eosinophils to guide the stepwise management and predict treatment response including clinical asthma outcomes particularly in patients with severe or uncontrolled asthma [12, 15, 22, 33]. However there is a lack of understanding in monitoring those levels among patients with clinical remission of asthma whether during treatment with ICS or before stepping down treatment.

Based on our results, the levels of FeNO and blood eosinophils were significantly decreased, and these decreases could predict that complete remission would follow. Regarding the population of the present study, the characteristics of our patients were considered to be clinical remission of asthma defined as a sustained absence of significant asthma symptoms (≥ 12 months) based on validated instruments and the optimization of their lung function. However, not only clinical remission, but also the resolution of airway inflammation is still considered a core requirement of definite complete remission. Previous studies found persisting airway inflammation among patients with clinical remission, whether receiving ongoing treatment with ICS or not taking any medication [34, 35]. Therefore, demonstrating

Table 4. Peripheral blood results and spirometry data according to a level of FeNO for all participants

	FeNO ≥ 25 ppb (n = 36)	FeNO < 25 ppb (n = 48)	p-value
Age (years), mean (SD)	54.5 (13.2)	55.4 (11.2)	0.740
Female, (%)	23 (63.8)	39 (81.3)	0.073
Mean body mass index (kg/m^2)	25.5 (23.8, 28.5)	26.1 (22.1, 28.7)	0.825
FVC (L), mean (SD)	2.6 (2.3, 2.8)	2.4 (1.9, 2.8)	0.508
FVC % predicted, mean (SD)	91.9 (11.5)	89.2 (16.7)	0.379
FEV_1 (L), mean (SD)	1.9 (0.5)	1.8 (0.6)	0.288
FEV_1 % predicted, mean (SD)	77.8 (10.7)	77.5 (18.1)	0.921
FEV_1/FVC (%), mean (SD)	0.71 (0.62, 0.75)	0.72 (0.65, 0.79)	0.975
PEF (LPM), mean (SD)	5.8 (1.6)	5.6 (1.9)	0.690
PEF % predicted, mean (SD)	92.4 (20.9)	93.8 (23.5)	0.769
Bronchodilator reversibility, (%)	5.5 (2, 12)	4 (0.25, 9)	0.508
Eosinophil (%)	6 (4, 8)	4 (3,6)	0.078
Eosinophil count (cell/mm^3)	450 (310, 602)	295 (190, 517)	0.123
Duration of asthma onset	10 (7, 20)	16 (3.3, 31.8)	0.508
Duration of well controlled asthma (month)	15.9 (8.7, 24.7)	12.5 (8.3, 20.20	0.270

Values are shown as median (interquartile range), mean (SD) or number (%). FeNO — fractional exhaled nitric oxide; FEV_1 — forced expiratory volume in 1 second; FVC — forced vital capacity; kg — kilogram; L — liter; LPM — liter per minute; m — meter; mm — millimeter; MMEF — maximum mid-expiatory flow; PEF — peak expiratory flow; ppb — parts per billion

Table 5. FeNO and spirometry data according to a level of blood eosinophil count for all participants

	Eosinophil ≥ 300 cell/mm3 (n = 54)	Eosinophil < 300 cell/mm^3 (n = 30)	p-value
Age, years	54.5 (45.7, 64)	56.5 (50.5, 640	0.378
Female, (%)	36 (66.7)	26 (86.6)	0.046
FeNO, ppb	27.5 (17, 44)	16.2 (10, 23.2)	0.003
Mean body-mass index (kg/m^2)	25.6 (23.1, 28.1)	25.8 (22.8, 29)	0.820
FVC (L), mean (SD)	2.6 (2.1, 3.2)	2.4 (1.9, 2.8)	0.111
FVC % predicted, mean (SD)	90 (80.7, 102.2)	89.5 (79.5, 98.7)	0.794
FEV_1 (L), mean (SD)	1.9 (1.4, 2.4)	1.7 (1.3, 2.1)	0.255
FEV_1 % predicted, mean (SD)	77 (67.7, 91)	80 (62.7, 89.5)	0.820
FEV_1/FVC (%), mean (SD)	0.71 (0.63, 0.78)	0.70 (0.63, 0.78)	0.948
PEF (LPM), mean (SD)	5.8 (4.5, 7.0)	5.7 (3.7, 6.8)	0.820
PEF % predicted, mean (SD)	95 (78.2, 108.2)	89 (73, 114.2)	0.820
Bronchodilator reversibility, (%)	4 (1, 10)	5.5 (2, 10.5)	0.820
Duration of asthma onset	11 (6, 30.2)	10.5 (4.7, 26.2)	0.820
Duration of well controlled asthma	13.8 (8.4, 18.9)	16.4 (9.3, 29.8)	0.495

Values are shown as median (interquartile range), mean (SD) or number (%). FeNO — fractional exhaled nitric oxide; FEV1 — forced expiratory volume in 1 second; FVC — forced vital capacity; kg — kilogram; L — liter; LPM — liter per minute; m — meter; mm — millimeter; MMEF — maximum mid-expiatory flow; PEF — peak expiratory flow; ppb — parts per billion

a current negative AHR and sputum eosinophilia are favorable components of establishing complete remission of asthma [36]. Although sputum induction and bronchial provocation testing were not been integrated into our study due to the difficulty in performing these tests and need

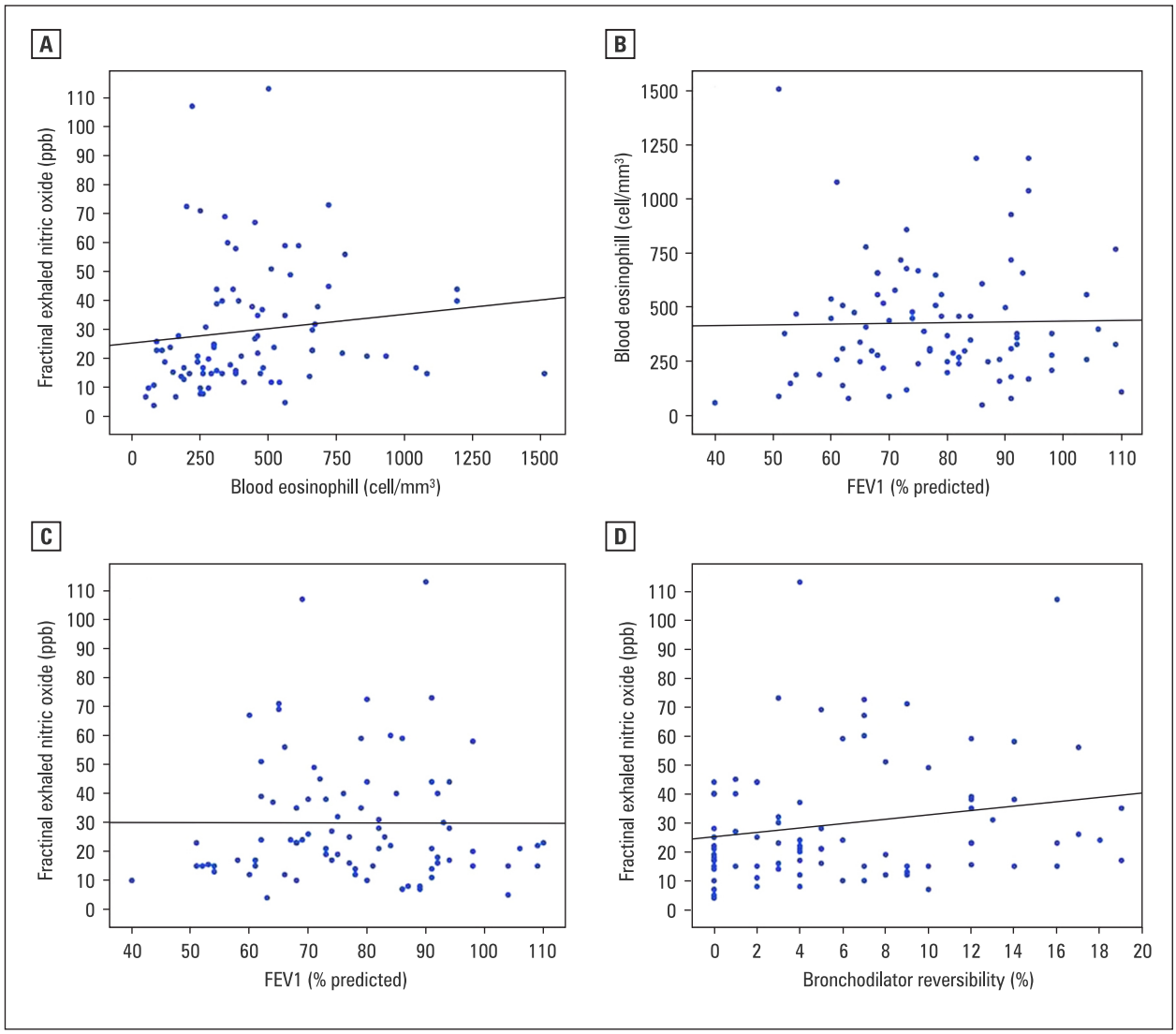

Figure 1. Scatter plots of correlation between biomarkers and lung function. **A)** Correlation between fractional exhaled nitric oxide and blood eosinophils (r = 0.310, p = 0.004); **B)** Correlation between fractional exhaled nitric oxide and FEV_1 %predicted (r = 0.003, p = 0.975); **C)** Correlation between blood eosinophils and FEV_1 %predicted (r = 0.035, p = 0.750); **D)** Correlation between fractional exhaled nitric oxide and bronchodilator reversibility (r = 0.203, p= 0.064)

for experts at specialist hospitals, the measurements of FeNO and blood eosinophils should be considered reliable alternative methods of determining airway inflammation and establishing the achievement of complete remission in our study. In this study, the detection of levels of FeNO and blood eosinophils below a reference cutoff value were seen, which indicates that our patients may have achieved complete remission from their treatments with ICS/LABA. Apart from testing based on FeNO and blood eosinophils being easier, more practical and more readily available methods, even in local hospitals, several studies have shown that either FeNO or blood eosinophils have a significant correlation with AHR and sputum eosinophils [37–41]. In addition, the author suggests that over a year in continuation of asthma medications could be indirectly reflected in the achievement. One previously-published study shows that the optimal AHR can be reached after more than a year of treatment with ICS [42, 43] and this has been associated with a reduction in the amount of inflammatory infiltrate [44]. Thus, the monitoring of FeNO and blood eosinophils may help clinicians to make a more informed decision on when and how to step down their treatments due to reflecting the improvement of airway inflammation, because both markers are represented as local and systemic inflammation, respectively. Additionally, a low level of FeNO in asymptomatic individuals, who are receiving ongoing asthma treatments, also suggests that they are receiving

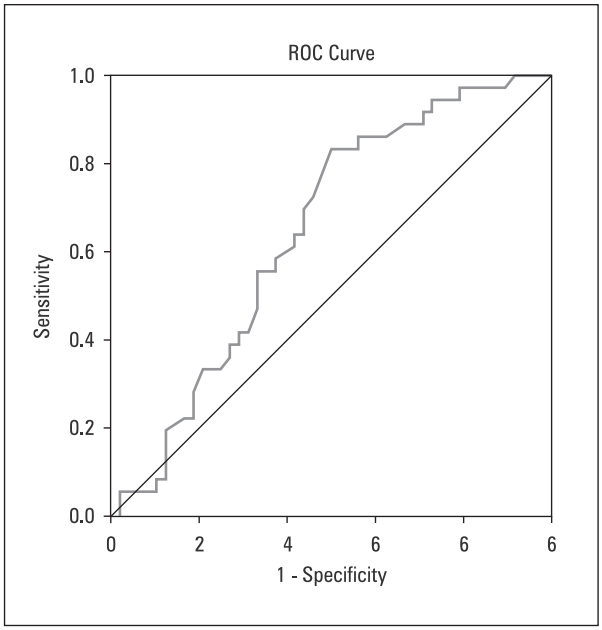

Figure 2. The receiver-operating characteristic curve analysis of the sensitivity and specificity of blood eosinophils for the identification of FeNO ≥ 25 ppb. The optimal cutoff point was 295 cell/mm^3 (sensitivity 83.5%, specificity 50%, AUC 64.4%, p = 0.024)

an adequate dosage of ICS, have good adherence, and have suitability for tapering ICS [12], while a low level of blood eosinophils is associated with less bad asthma-related outcomes [33].

The present study has shown that there was a significant correlation between FeNO and blood eosinophils. This relationship may be established in asthmatic patients with complete remission who are taking low-dose ICS/LABA. Variations have been found in the relationship between FeNO and blood eosinophils among different asthmatic patients. In contrast, a study of Hanh et al. showed that FeNO was well correlated with blood eosinophil levels (r = 0.5217, p = 0.0004) among children with uncontrolled asthma [45], whereas Gao et al. found no relationship between these two variables in uncontrolled adult asthma (p = 0.5801) [19]. In patients with partly controlled asthma, Badar et al. demonstrated a positive correlation between FeNO and blood eosinophils (r = 0.276, p = 0.017) [46]. In addition, the current study observed no correlation between either FeNO or blood eosinophils and lung functions in patients with clinical remission. However, the previous study also demonstrated that spirometry and FeNO have no significant correlation [47]. These findings may indicate that the airway caliber should be maximized and stable after the achievement of lightening airway inflammation with long duration treatment by ICS/LABA. In contrast, one recent report suggests that FeNO and blood eosinophils are significantly elevated with poorer lung functions in patients with uncontrolled asthma [48]. In this study, the cutoff value of 295 cell/mm3 for blood eosinophils could predict the value of < 25 ppb for FeNO in the clinical remission of asthma. These results could be important in the assessment of airway inflammation in settings where FeNO measurement is not available. Future studies may prove fruitful validation of blood eosinophil cutoff values that determine inactive eosinophilic airway inflammation in patients with well-controlled asthma.

The limitations of our study are mainly due to its structure as a unicentric study, the findings of which may not have external validity. Neither sputum induction for determining sputum eosinophilia nor bronchial provocative tests for establishing AHR were integrated into the current study, because these procedures are too sophisticated and carry a risk of complications during processing. Another limitation of this study is its cross-sectional approach. Because a significant variability in blood eosinophil counts and FeNO for determining airway inflammation has been reported [49, 50], a single measurement of both biomarkers may not provide a reliable enough value for the interpretation of achieved airway inflammation in patients with clinical remission of asthma. A longitudinal study with multiple measurements of biomarkers would be reasoned as a way to resolve this weakness.

In conclusion, this study could potentially support the role of FeNO and eosinophil measurements for determining the status of airway inflammation and confirming complete remission in patients with clinical remission of asthma. Moreover, the level of blood eosinophils may serve as a potential biomarker for the prediction of the level of FeNO in those patients.

Clinical Trial Registry

NCT04454385 at Clinicaltrials.gov.

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Author contributions

N.N. had full access to all of the data in the study and takes responsibility for the integrity of the data, accuracy of the data analysis, study

design, data analysis and interpretation, and the writing of the manuscript. T.R., T.P. and P.T. take responsibility for study design, data analysis and interpretation.

Conflict of interest

None declared.

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