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Relationship of fractional exhaled nitric oxide with blood eosinophilia in characterizing type-2 airway inflammation in treatment-naïve patients with chronic obstructive pulmonary disease

Riksoam Chatterjee,[1] Mansi Gupta,[1] Zia Hashim,[1] Ajmal Khan,[1] Alok Nath,[1] Vikas Aggarwal[2]

[1]Department of Pulmonary Medicine, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow; [2]Department of Immunology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India

Abstract

Fractional exhaled nitric oxide (FeNO), a sensitive and reproducible, non-invasive biomarker for type-2 (T2) inflammation in asthma, remains underutilized in chronic obstructive pulmonary disease (COPD). We investigated the potential role of FeNO and its relationship with blood eosinophilia in characterizing T2 airway inflammation in COPD. A single-center prospective observational study was conducted in 75 treatment-naïve adult patients with stable COPD and 75 age-sex-matched controls. The Global Initiative for Chronic Obstructive Lung Disease criteria were used for the diagnosis of COPD. FeNO levels were compared with clinico-radiological features, pulmonary functions, and other markers of T2 inflammation in COPD. Participants in the COPD subgroup had a median age of 62 (55.0, 69.0) years with a male predominance (77.3%). The median FeNO value was 27.0 ppb (19.0, 39.0) in COPD and 14.0 ppb (10.0, 20.0) in controls ($p<0.001$). FeNO values were categorized as low (≤25 ppb), intermediate (25-50 ppb), and high (≥50 ppb), with 57.3% of COPD patients having intermediate to high FeNO. In contrast, 46.7% of these patients had an absolute eosinophil count (AEC) ≥300/mm³. Higher values of FeNO in COPD were associated with the history of atopy ($p<0.001$), a positive bronchodilator reversibility on spirometry ($p<0.05$), and high-resolution computed tomography findings such as emphysema, bronchial wall thickening, bronchiectasis/bronchiolectasis, and mosaic attenuation ($p<0.05$). In addition, FeNO levels showed a strong positive linear correlation with serum immunoglobulin E (IgE) levels ($r^2=0.66$, $p<0.001$) and AEC ($r^2=0.56$, $p<0.001$). FeNO's best diagnostic cut-off level to detect T2 inflammation was 31 ppb (sensitivity: 65.9%, specificity: 86.5%; area under the receiver operating characteristic curve: 0.79). FeNO measurement, besides blood eosinophilia, is an effective, direct, airway-specific, non-invasive tool for detecting T2 airway inflammation in stable patients with COPD. Atopy (IgE sensitization) is a crucial factor in explaining higher FeNO values in COPD patients. FeNO correlates well with blood eosinophilia in COPD patients with an eosinophilic phenotype as a surrogate biomarker with good sensitivity and specificity.

Key words: fractional exhaled nitric oxide, chronic obstructive pulmonary disease, type-2 airway inflammation, eosinophilic inflammation.

Correspondence to: : Mansi Gupta, Department of Pulmonary Medicine, IVth Floor, Pradhan Mantri Swasthya Suraksha Yojana Block, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India-226014. Tel.: +919811423197. E-mail: drmansipccm@gmail.com

Introduction

Chronic obstructive pulmonary disease (COPD) is a global health concern, projected to become the third leading cause of death by 2030 [1,2]. Its prevalence is increasing due to aging populations and rising exposure to risk factors, particularly in low- and middle-income countries [2]. COPD is characterized by chronic airway inflammation, structural lung changes, and progressive airflow limitation that is often irreversible [3]. Traditionally, COPD inflammation was attributed to type-1 immune responses, driven by neutrophils, CD8+ T-cells, and macrophages [4-7]. However, recent evidence suggests that a subset of COPD patients also exhibit type-2 (T2) inflammation, which is typically associated with asthma and involves eosinophils, Th2 cells, and T2 innate lymphoid cells (ILC2s) [8-13].

Persistent blood eosinophilia (≥2%) is observed in 30–40% of COPD patients, suggesting a significant eosinophilic component [11]. Elevated blood eosinophil levels are associated with a higher risk of exacerbations [14,15] and predict better responses to inhaled corticosteroids (ICS) [16,17]. Additionally, persistent eosinophilia correlates with accelerated lung function decline, reinforcing its role as a clinically relevant inflammatory phenotype [18]. Given these findings, airway eosinophilia has been proposed as a treatable trait in COPD, highlighting the need for precise biomarkers to guide personalized treatment strategies [12,13]. Biomarkers reflecting T2 inflammation, such as blood eosinophilia, sputum eosinophilia, and fractional exhaled nitric oxide (FeNO), are critical in clinical decision-making. While their utility is well established in asthma [19,20], their role in COPD remains controversial due to inconsistencies in defining appropriate thresholds for T2 inflammation [21,22]. Peripheral blood eosinophil counts [absolute eosinophil

counts (AECs)] are commonly used as a surrogate marker for airway eosinophilia in COPD [23]. However, their correlation with sputum, tissue, and bronchoalveolar lavage (BAL) eosinophils varies, and levels fluctuate due to systemic factors such as medication use, malignancies, and parasitic infections, especially in tropical and subtropical regions [24-28]. This variability underscores the need to explore additional biomarkers beyond blood eosinophilia to improve the identification of T2 airway inflammation in COPD.

FeNO is a non-invasive, reproducible, and readily available biomarker that measures T2 inflammation directly from exhaled breath [29,30]. It is widely recommended for diagnosing and monitoring asthma [30-32], but its role in COPD remains unclear due to inconsistent findings. Limited studies, particularly in Southeast Asia, have investigated its clinical relevance in COPD [33]. The interpretation of FeNO in COPD is complicated by smoking status, as active smoking suppresses FeNO levels due to increased oxidative stress and nitric oxide synthase inhibition. Therefore, current smokers have artificially lower FeNO levels than nonsmokers or ex-smokers, which affects its clinical applicability in COPD patients with a smoking history [29,30]. Additionally, the literature suggests that the presence of atopy/allergic sensitization, also indicated by elevated serum immunoglobulin E (IgE) levels, is observed in roughly one-third of COPD patients [34] and could be a key driver of elevated FeNO levels in COPD.

Donohue *et al.* have reported increased FeNO levels (≥25 ppb) in 8% of COPD patients, particularly those with asthma-COPD overlap (ACO) [35], while Chen *et al*. identified >22.5 ppb FeNO as a potential cut-off for differentiating ACO from COPD [36]. A recent meta-analysis demonstrated mild FeNO elevation in stable COPD [33], with higher levels correlating with increased ICS responsiveness [37]. Additionally, a systematic review indicated that FeNO levels significantly decreased with corticosteroid treatment in ex-smokers with COPD [38]. Keeratichananont *et al.* found that FeNO strongly correlates with blood eosinophilia and may predict poor clinical outcomes in COPD [39]. Based on the conflicting results from previous studies, the exact role of FeNO measurement in evaluating and managing COPD remains to be further investigated. The primary objective of this study was to explore the utility of measuring FeNO as an inflammatory biomarker for T2 airway inflammation in stable patients with COPD.

Materials and Methods

A prospective single-center observational study was conducted at a tertiary care hospital in India between March 2023 and February 2024. 75 adult patients with stable COPD were enrolled. The study was conducted after approval was obtained from the Institutional Ethics Committee. All participants signed a written informed consent to participate in this study before enrollment. The diagnosis and assessment of COPD were performed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria, 2023 [40]. Newly diagnosed, treatment-naïve, stable adults (aged 40 years and older) diagnosed with COPD were considered eligible for enrollment in this study. Patients who were either newly diagnosed or had never received any long-term maintenance pharmacological therapy for COPD, including long-acting bronchodilators, inhaled or systemic corticosteroids, and theophyllines, were included as treatment-naïve patients. Patients with other chronic obstructive lung conditions, including but not limited to asthma, bronchiectasis, allergic bronchopulmonary aspergillosis, cystic fibrosis, bronchiolitis, and reactive airway dysfunction syndrome, were excluded. Patients were also excluded if they were current smokers or had acute or chronic respiratory failure, other causes of peripheral blood eosinophilia, recent respiratory tract infection within the last 6 weeks, or recent use of corticosteroids (oral or inhaled) in the previous 6 weeks. This study also included equal age-matched and sex-matched controls (Figure 1). The base-

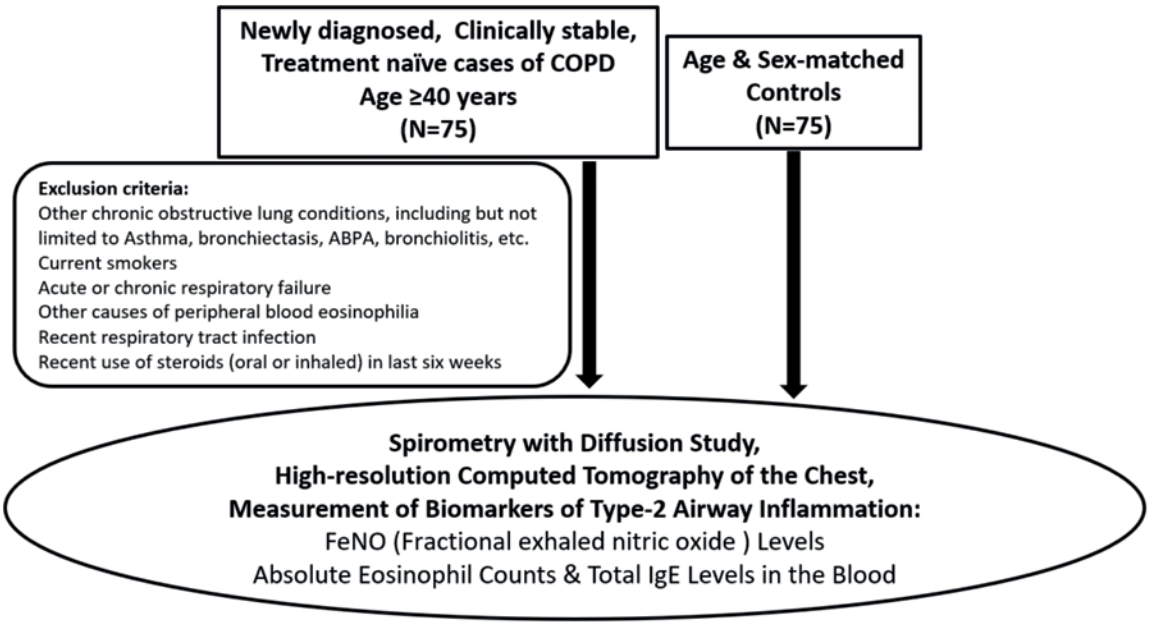


Figure 1. Study workflow diagram. ABPA, allergic bronchopulmonary aspergillosis; COPD, chronic obstructive pulmonary disease; FeNO, fractional exhaled nitric oxide.

line characteristics of the participants, including demographic information, history of smoking status and other exposures, and relevant medical information, were obtained. Subjects underwent measurement of various biomarkers of T2 inflammation, including FeNO levels, blood levels of eosinophils, and total IgE. Spirometry and diffusion capacity (DLCO) measurements were performed for all the subjects. In addition, the COPD group underwent a detailed radiological assessment of the chest using high-resolution computed tomography (HRCT).

Measurement of inflammatory biomarkers

The measurement of FeNO levels was performed according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations *via* a hand-held, portable, battery-operated electrochemical breath analyzer (Medisoft, Belgium; designed, developed, and manufactured by validated Bedfont® Scientific Limited) [31]. The investigators underwent adequate training on the device before the initiation of the study to perform FeNO measurements according to the guidelines. Exhalations were performed at 10-20 cm H_2O pressure to maintain a flow rate of 50 mL per second. A visual incentive was activated upon exhalation to assist patients in maintaining a constant flow rate. The device ensured that expiration efforts below the fixed flow rate requirements did not increase the FeNO value. Each patient was allowed up to three attempts to perform a valid FeNO measurement. The FeNO values are reported as parts per billion (ppb).

Peripheral venous blood samples were taken to measure blood eosinophilia, and the results are expressed as the absolute AEC per cubic millimeter and the percentage of total leukocyte count (E%). The levels of total IgE in the serum were assayed *via* commercial enzyme-linked immunosorbent assay-based kits according to the manufacturer's instructions. The values of total IgE were expressed as International Units per milliliter (IU/mL).

Spirometry and diffusion capacity measurements

Pulmonary function tests (PFTs) with bronchodilator reversibility and DLCO measurements were performed *via* the Ganshorn Power Cube-Body plus Diffusion system (based on ultrasound technology, with LFX software for built-in quality control monitoring) by well-trained professional staff at our PFT unit. The PFTs followed the latest ATS-ERS recommendations [41-43]. The lung function parameters collected included forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), pre- and post-bronchodilator FEV1/FVC ratios, forced maximal mid-expiratory flows, and DLCO values. The presence (≥10% of the predicted value for either FEV1 or FVC) of bronchodilator reversibility was also noted for the study subjects [41,42].

Radiological assessment

The study group comprising COPD patients underwent volumetric thin-section computed chest tomography with HRCT to adequately assess the lung parenchyma and airways. Structural changes compatible with COPD were recorded, including emphysema, bullae, bronchial wall thickening, bronchiectasis, and bronchiolectasis. The radiological features suggestive of small airway involvement, such as centrilobular nodules, mosaic attenuation, and air trapping (on expiratory computed tomography performed at functional residual capacity or residual volume), were also recorded.

Statistical analysis

STATA 18.0 software (Stata Corp. 2019; Stata Statistical Software: Release 18. StataCorp LLC, College Station, TX, USA) was used for statistical analysis. To achieve a power of 80% and an α of 0.05, Cohen's d of 0.5 for a medium effect size, the sample size required was 64 in each group. Assuming an attrition of 10%, we rounded it to 75 in each group. The normality assumption for continuous variables was tested *via* the Shapiro-Wilk test; however, the normality hypothesis was rejected. Descriptive statistics (median, interquartile range) were used to present demographic and clinical (continuous) assessment information. Data with proportions are expressed as frequencies and percentages. The Kruskal-Wallis test for two-group comparisons between cases and controls was used to compare the continuous variables. Similarly, the Kruskal-Wallis analysis of variance test was used for comparisons between patient characteristics and subgroups of FeNO levels ($FeNO_{low}$ <25 ppb, $FeNO_{intermediate}$ ≥25-50 ppb, and $FeNO_{high}$ ≥50 ppb). Fisher's exact test was used to compare the categorical variables. Using Spearman's correlation method, FeNO levels were tested for strength and direction of correlation with peripheral eosinophilia. We used the COPD prevalence of 7% in our area to calculate the negative and positive predictive values [44]. Receiver operating characteristic (ROC) curves with areas under the curve (AUCs) were plotted for FeNO to AECs ≥300/mm³ and AECs ≥150/mm³ *via* the Henry and McNeil method. The optimal cut-off value, sensitivity, specificity, and Youden index were also calculated. Statistical significance was considered at $p<0.05$.

Results

Characteristics of the study participants

A total of 75 adult stable treatment-naïve patients with COPD (according to the inclusion and exclusion criteria) were enrolled in the study group and compared with an equal number (75) of age- and sex-matched individuals in the control arm. The baseline clinico-demographic profile of the study participants is given in Table 1. The study population of 150 subjects comprised predominantly males (76.6%), including 75 COPD patients (77.3% males), with a median age of 62 [95% confidence interval (CI): 59.0-64.0] years, who were mainly from urban areas (65.3%). Comorbidities were significantly higher in COPD patients (61.3% *vs*. 32% in controls, $p<0.001$), with hypertension (40%), diabetes (28%), cardiovascular disease (22%), hypothyroidism (10%), chronic liver/kidney diseases (8%), and cancers (5%) being the most prevalent. Chronic respiratory conditions were excluded. According to the GOLD 2023 severity classification [39], the COPD patients were classified into GOLD Group 2 (57.3%) and Group 3 (32%), whereas 10.6% of the patients were in Group 4. The patient group consisted of former smokers (70.7%) and never-smokers (29.3%) based on their smoking history or significant exposure to biomass fuel (≥10 years). Current smokers were excluded from the study to avoid inaccurate interpretation of FeNO values. The PFT values and serum levels of biomarkers of T2 inflammation were compared between COPD patients and controls, with statistically significant differences ($p<0.001$).

The median baseline values of FeNO, AEC, E%, and IgE levels in the COPD subgroup were 27 ppb (95% CI: 23.5-31.0), 268/mm³ (95% CI: 220.0-355.2), 3% (95% CI: 2.0-4.0), and 461.0 IU/mL (95% CI: 324.2-765.4), respectively. Up to 46.7% of COPD patients had AECs ≥300/mm³, suggesting ongoing eosinophilic airway inflammation [39]. AECs ≥150/mm³ were noted in up to 81.3% of

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Table 1. Baseline characteristics of study subjects.

Parameters	Cases (n=75)	Controls (n=75)
Age (years)	62.0 (59.0-64.0)	59.0 (55.0-62.5)
Males	58 (77.3%)	57 (76.0%)
BMI (kg/m^2)	24.4 (23.2-25.3)	24.0 (23.0-25.1)
Urban background	49 (65.3%)	42 (56.0%)
Smoking/BMFE		
Former	53 (70.7%)*	0 (0%)
Never	22 (29.3%)	75 (100%)
Comorbidities	46 (61.3%)*	24 (32%)
Lung functions		
Post-bronchodilator FEV1/FVC (%)	0.66 (0.63-0.68)*	0.93 (0.92-0.95)
FVC (L)	1.94 (1.77-2.01))*	2.98 (2.96-3.08)
FVC percentage predicted (%)	59.0 (56.0-67.0)*	86.0 (83.5-86.5)
FEV1 (L)	1.31 (1.08-1.43)*	2.77 (2.74-2.94)
FEV1 percentage predicted (%)	52.0 (45.5-55.5)*	84.0 (81.5-85.0)
Bronchodilator reversibility	17 (22.7%)*	0 (0.0%)
DLCO percentage predicted (%)	53.0 (49.5-60.0)*	81.0 (80.0-85.0)
VA (L)	3.1 (2.5-3.6)*	4.4 (3.9-5.2)
KCO (mmol/min/kPa/L)	1.1 (0.9-1.4)	1.3 (1.1-1.4)
Markers of type-2 inflammation		
Blood eosinophilia (%)	3.0 (2.0-4.0)*	2.0 (1.8-2.4)
Absolute blood eosinophil counts (/mm3)	268.0 (220.0-355.2)*	160.0 (140.3-189.0)
Serum total IgE (IU/mL)	461.0 (324.2-765.4)*	133.0 (109.5-187.0)
FeNO levels (ppb)	27.0 (23.5-31.0)*	14.0 (13.0-15.6)

BMI, body mass index; BMFE, biomass fuel exposure; DLCO, diffusion capacity of lungs for carbon monoxide; FVC, forced vital capacity; FEV1, forced expiratory volume over 1st second; FENO, fractional exhaled nitric oxide; IQR, interquartile range; IgE, immunoglobulin E; KCO, carbon monoxide transfer coefficient; ppb, parts per billion; VA, alveolar volume. Data are expressed as medians with 95% confidence intervals. *p<0.05 *vs.* controls.

patients in the COPD subgroup. Conversely, FeNO levels ≥25 ppb were detected in 57.3% of patients with COPD; however, high FeNO levels ≥50 ppb were detected in only 9.3% of patients. Interestingly, the proportions of COPD patients with either FeNO levels ≥25 ppb or AECs ≥300/mm^3 were 23.9% and 13.3%, respectively. Additionally, 33.4% of COPD patients had an AEC ≥300/mm^3 along with intermediate to high FeNO levels ≥25 ppb.

Comparison of patient characteristics among groups with low, intermediate, and high fractional exhaled nitric oxide levels

FeNO values were categorized as low (<25 ppb), intermediate (≥25-50 ppb), or high (≥50 ppb) in the COPD group, as summarized in Table 2. The numbers of COPD patients with $FeNO_{low}$, $FeNO_{intermediate}$, and $FeNO_{high}$ were 32 (42.6%), 36 (48%), and 7 (9.4%), respectively. No significant differences among these three FeNO groups were observed in baseline clinico-demographic characteristics or spirometric values, except for the presence of a history of atopy (p<0.001) and bronchodilator reversibility (p=0.049). However, statistically significant differences (p<0.001) were observed in the markers of T2 airway inflammation among patients with low, intermediate, and high levels of FeNO (Table 2). In addition, the comparison of various radiological findings on the HRCT chest among the three FeNO groups revealed significant differences in the presence of emphysema (p=0.024), bronchial wall thickening (p=0.05), and bronchiolectasis or bronchiectasis (p=0.041). Interestingly, the presence of mosaic attenuation or air trapping seen on expiratory scans *via* HRCT, suggestive of small airway disease, significantly differed among the three FeNO groups (p<0.001).

Diagnostic accuracy of the fractional exhaled nitric oxide in defining type-2 airway inflammation in chronic obstructive pulmonary disease patients

Peripheral eosinophilia currently serves as a surrogate for indicating eosinophilic airway inflammation in patients with COPD [31,39]. The mean FeNO levels were correlated with AEC and E%, which are measures of peripheral eosinophilia. Spearman's correlation analysis revealed a positive correlation between FeNO level and markers of peripheral eosinophilia (AEC and E%). Spearman's correlation coefficients for FeNO levels with AEC (Figure 2A) and E% in peripheral blood were 0.557 (p<0.001) and 0.446 (p<0.001), respectively. In addition, the serum IgE level, a surrogate marker of atopy and T2 inflammation, showed a strong positive correlation (Spearman's correlation coefficient, 0.664; p<0.001) with FeNO levels (Figure 2B). The constructed ROC curves defined the optimal cut-off value for the FeNO concentration for evaluating eosinophilic airway inflammation in COPD patients. The best diagnostic cut-off level of FeNO for detecting T2 airway inflammation, considering an AEC ≥300/mm^3, was 31 ppb (sensitivity 65.9%, specificity 86.5%, Youden index 0.52, and a negative predictive value of 96.6%), with the ROC-AUC estimated at 0.79 (Figure 3A). On the other hand, the estimated ROC-AUC was 0.73 for FeNO for AECs ≥150/mm^3, with an optimum cut-off value of 21 ppb (sensitivity 73.8%, specificity 64.3%, Youden index 0.38) (Figure 3B).

Discussion

COPD is a heterogeneous inflammatory airway disease in which inhaled irritants recruit and activate innate inflammatory cells in the

lungs and airways, cause tissue destruction, and disrupt normally functioning lung repair mechanisms [4-6]. While neutrophilic inflammation dominates the airway inflammatory pathway in most COPD patients [7], eosinophilic inflammation, as a directly treatable trait, has recently garnered attention in COPD patients over the last few years [9-14,21]. Blood eosinophilia is a practical surrogate for assessing T2 inflammation in COPD, but concerns about its reliability as a biomarker [24-27] highlight the need to explore alternatives like FeNO [22,29,30]. This prospective observational study evaluated FeNO as a potential marker of T2 airway inflammation in treatment-naive, stable COPD patients. The results indicate that elevated FeNO levels (≥25 ppb) are common in stable COPD, observed in 57.3% of patients, especially among those with atopy and eosinophilic inflammation as prominent features of their disease. It is noteworthy from our results that the history of atopy in COPD (18.6% in this study) is associated with higher FeNO levels in these patients. Allergic sensitization (atopy) has been linked not only to a higher risk of developing asthma later in life but also to the degree of airflow limitation, a key determinant of COPD in late adulthood [34]. Its role in eosinophilic or T2 inflammation-driven COPD may be more than coincidental and needs to be explored further. Furthermore, FeNO levels correlate well with other markers of T2 airway inflammation, such as blood eosinophilia and serum IgE levels. At an optimal cut-off of 31 ppb, the FeNO measurement demonstrated a reasonable diagnostic accuracy for T2 inflammation, when defined by AECs ≥300/mm^3. Our findings echo those of several previously published studies on the use of FeNO in COPD patients [33-36]. Consistent with the findings of earlier studies, our results further reinforce the adjunctive utility of FeNO in managing COPD patients, particularly in its ability to identify those with T2 airway inflammation.

Interestingly, this study has revealed that up to one-fourth of patients with COPD had FeNO levels ≥25 ppb with AECs below 300/mm^3. On the other hand, 13% of patients had AECs ≥300/mm^3 but low FeNO levels. These findings indicate that relying solely on eosinophil counts to identify eosinophilic inflammation in COPD patients may be insufficient. Recently, a study by Liu *et al.* investigated the role of combining FeNO and blood eosinophil count in COPD. This study revealed that combined measurement of eosinophils and FENO levels strengthened their ability to predict acute exacerbations. Combining these two biomarkers may provide a comprehensive understanding of COPD heterogeneity and offer precise guidance for making informed clinical decisions [45]. The 2024 GOLD guidelines suggest using an ICS for COPD patients with an eosinophil count greater than 100, a threshold often exceeded in the general population [28]. This criterion alone may not be the most reliable indicator of T2 airway inflammation, especially in India, where eosinophil counts are typically relatively

Table 2. Comparison of patient characteristics among groups with low, intermediate and high fractional exhaled nitric oxide levels.

Parameters	$FeNO_{low}$ ≤25 ppb (n=32)	$FeNO_{intermediate}$ >25 to <50 ppb (n=36)	$FeNO_{high}$ ≥50 ppb (n=7)
Age (years)	62.0 (58.0-65.0)	60.0 (56.2-65.6)	65.0 (45.3-74.4)
Male	26 (81.2%)	28 (77.8%)	4 (57.1%)
BMI (kg/m^2)	23.8 (22.1-24.9)	25.5 (23.9-27.0)	22.4 (21.2-29.8)
Smoking/BMFE			
Former	23 (71.9%)	27 (75%)	3 (42.9%)
Never	9 (28.1%)	9 (25%)	4 (57.1%)
History of atopy	1 (3.1%)**	8 (22.2%)	5 (71.4%)
CAT score	17.0 (15.0-19.0)	15.0 (14.0-16.0)	14.0 (11.3-22.4)
Lung Functions			
Post-bronchodilator FEV1/FVC (%)	0.66 (0.61-0.68)	0.66 (0.63-0.69)	0.66 (0.56-0.69)
FVC (L)	1.90 (1.66-2.01)	2.00 (1.78-2.14)	1.60 (0.71-2.82)
FVC percentage predicted (%)	66.5 (51.0-70.0)	58.0 (54.4-65.0)	69.0 (45.2-72.0)
FEV1 (L)	1.20 (1.04-1.38)	1.40 (0.99-1.59)	1.02 (0.56-1.89)
FEV1 percentage predicted (%)	49.0 (45.0-55.0)	53.5 (344.4-58.8)	58.0 (38.7-65.1)
$MEF_{25-75\%}$ percentage predicted (%)	22.5 (19.2-33.1)	27.0 (20.2-32.4)	24.0 (15.9-48.1)
Bronchodilator reversibility	6 (18.8%)*	14 (38.9%)	4 (57.1%)
DLCO percentage predicted (%)	48.0 (40.1-60.1)	58.5 (51.2-62.8)	55.0 (31.9-72.5)
Radiological findings (HRCT chest)			
Emphysema	26 (81.2%)*	25 (69.4%)	2 (28.6%)
Bullae	4 (12.5%)	8 (22.2%)	0 (0%)
Bronchial wall thickening	17 (53.1%)*	28 (77.8%)	6 (85.7%)
Bronchiolectasis/bronchiectasis	8 (25%)*	19 (52.8%)	4 (57.1%)
Centrilobular nodules	6 (18.8%)	8 (22.2%)	4 (57.1%)
Mosaic attenuation/air-trapping	3 (9.4%)**	20 (55.6%)	6 (85.7%)
Markers of type-2 inflammation			
Blood eosinophilia (E%)	2.0 (1.5-3.0)*	3.0 (2.0-4.0)	6.0 (3.0-8.4)
AEC (/mm^3)	210.0 (156.0-270.0)**	340.0 (240.0-440.7)	510.0 (277.7-897.9)
Serum total IgE (IU/mL)	202.9 (160.0-412.2)**	683.0 (349.2-947.2)	1222.0 (951.4-1406.0)

AEC, absolute eosinophil count; BMI, body mass index; BMFE, biomass fuel exposure; CAT, chronic obstructive pulmonary disease assessment test; DLCO, diffusion capacity of lungs for carbon monoxide; FVC, forced vital capacity; FEV1, forced expiratory volume over 1st second; FeNO, fractional exhaled nitric oxide; ppb, parts per billion; IgE, immunoglobulin E; IQR, interquartile range; $MEF_{25-75\%}$, maximal mid-expiratory flows; HRCT, high-resolution computed tomography. Data are expressed as medians with 95% confidence intervals. *p<0.05 *vs.* other FeNO groups; **p<0.001 *vs.* other FeNO groups.

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high due to prevalent parasitic infections, environmental stimuli, and other eosinophilic disorders [46,47]. Based on the findings of our study, we are confident that combining FeNO with AEC (blood eosinophilia) will give a more accurate prediction of T2 airway inflammation in COPD. This approach will reduce the confounding factors responsible for peripheral eosinophilia, especially in tropical and subtropical populations, where peripheral eosinophilia is highly prevalent. Identifying eosinophilic inflammatory components in COPD by combining various biomarkers may lead to more effective treatment choices in this heterogeneous disease process, helping to select patients who would most benefit from therapies, including biologics [48-50].

We have also compared the radiological findings on HRCT chest images among three groups of COPD patients with low, intermediate, and high FENO levels, revealing significant differences in radiological manifestations such as emphysema, bronchial wall thickening, bronchiolectasis, and bronchiectasis. Notably, mosaic attenuation or air trapping on expiratory HRCT scans, indicative of small airway disease, significantly differed across the three FeNO groups ($p<0.001$). This finding emphasizes that FeNO is a relatively more airway-centric marker of T2 inflammation. Combining FeNO with AEC in the blood may increase the diagnostic value for predicting small airway involvement in COPD and requires further investigation.

The results of our study are exceptionally reliable, considering our robust methodology and the inclusion criteria of only nonsmok-

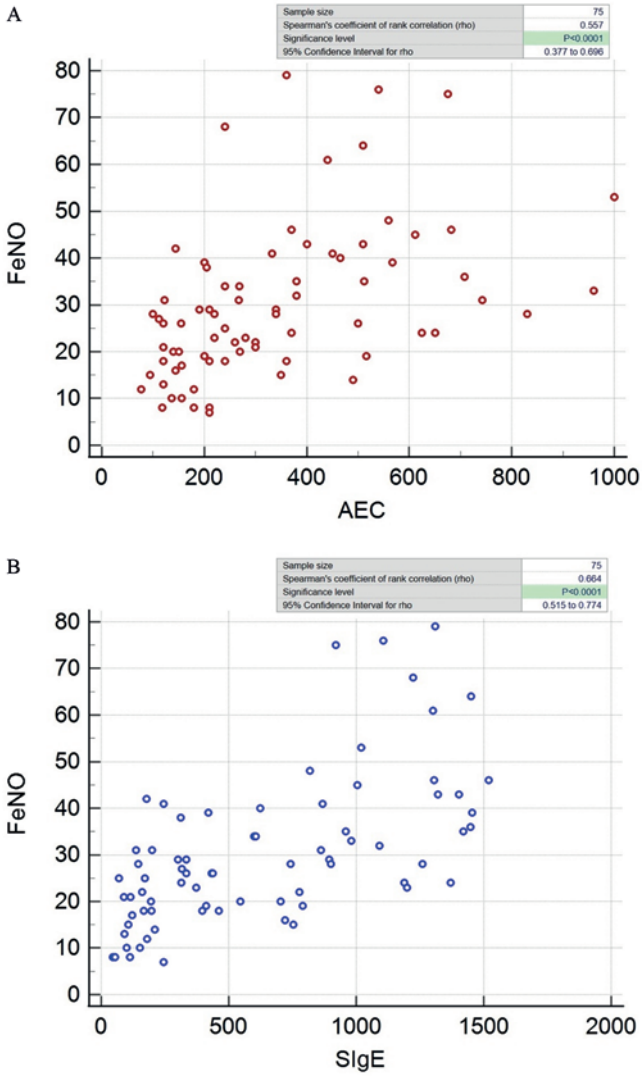


Figure 2. A) Correlation of mean fractional exhaled nitric oxide (FeNO) levels (ppb) with peripheral absolute eosinophil cell count (AEC) per cubic millimeter of blood in stable patients with chronic obstructive pulmonary disease (COPD) determined via Spearman's correlation coefficient; B) correlation of mean FeNO level (ppb) with the serum immunoglobulin E (SIgE) levels (IU/mL) in stable patients with COPD determined via Spearman's correlation coefficient.

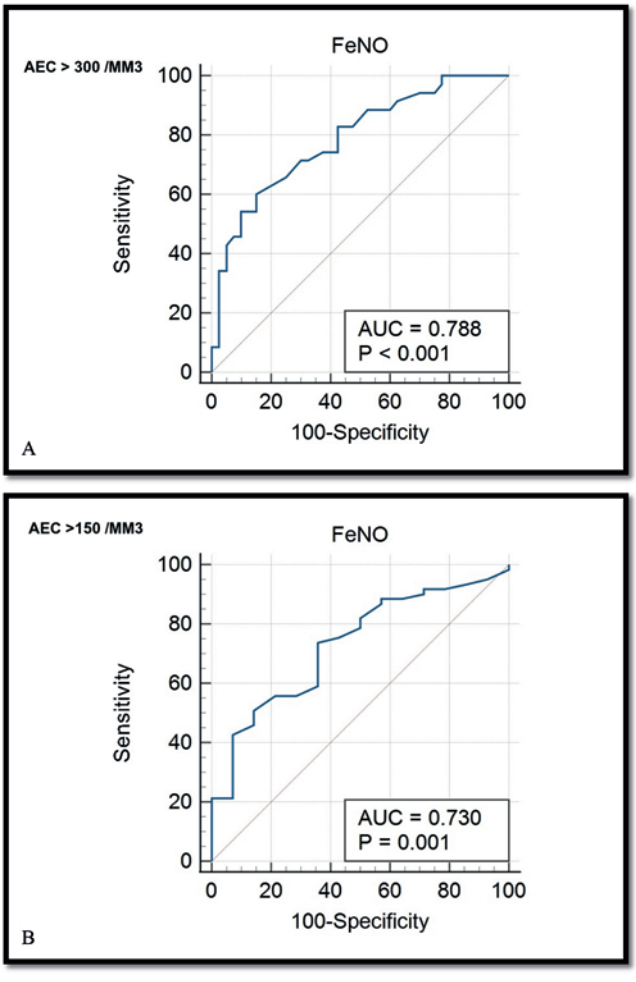


Figure 3. A) The receiver operating characteristic (ROC) curve defines the optimal cut-off level of FeNO (>31 ppb) for diagnosing T2 airway inflammation, as indicated by the area under the curve (AUC) values for an absolute eosinophil count (AEC) ≥300/mm^3; B) the ROC curve defines the optimal cut-off level of FeNO (>21 ppb) for diagnosing T2 airway inflammation, as indicated by AUC values for an AEC ≥150/mm^3.

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ers and ex-smokers for our COPD group. The smoking status of a patient has a significant impact on FeNO results in COPD, with current smokers exhibiting artificially lower FeNO levels compared to nonsmokers or ex-smokers [29-31]. However, this study also has a few limitations. This was a single-center study performed in a limited cohort of patients, and a larger sample size would provide more certainty to the results. Additionally, for comparison, our study did not utilize other biomarkers of eosinophilic inflammation, such as sputum or BAL eosinophils. In summary, this study demonstrated that FeNO is a simple, noninvasive, and effective tool for identifying T2 airway inflammation in treatment-naive, stable COPD patients. FeNO correlates well with blood eosinophilia in COPD patients with an eosinophilic phenotype, serving as a surrogate biomarker with good sensitivity and specificity. Atopy (IgE sensitization) appears to be a key factor in elevated FeNO levels in patients with COPD. While FeNO levels can vary in COPD, their diagnostic value may improve when used in carefully selected patients.

Conclusions

The study highlights the diagnostic utility of FeNO as a direct, airway-specific, non-invasive, and feasible biomarker for evaluating T2 airway inflammation in stable, treatment-naïve COPD patients. FeNO correlates well with blood eosinophilia in COPD patients with an eosinophilic phenotype, serving as a surrogate biomarker with good sensitivity and specificity. Combining FeNO measurements with eosinophil counts may enable more precise and individualized decisions on when to initiate ICS therapy. Adding FeNO to the management approach for COPD, aimed at targeting early detection of eosinophilic inflammation, could also support the use of targeted biologic therapies, potentially transforming treatment strategies for eosinophilic COPD with persistent symptoms. However, additional large-scale prospective studies are needed to further establish the role of FeNO in COPD, which may have implications for personalized, targeted management strategies in these patients.

References

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006;3:e442.
2. World Health Organization. The top 10 causes of death. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.
3. Tamondong-Lachica DR, Skolnik N, Hurst JR, et al. GOLD 2023 update: implications for clinical practice. Int J Chron Obstruct Pulmon Dis 2023;18:745-54.
4. Kim V, Rogers TJ, Criner GJ. New concepts in the pathophysiology of chronic obstructive pulmonary disease. Proc Am Thorac Soc 2008;5:478-85.
5. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2016; 138:16-27.
6. Agustí A, Hogg JC. Update on the pathogenesis of chronic obstructive pulmonary disease. N Engl J Med 2019;381: 1248-56.
7. Butler A, Walton GM, Sapey E. Neutrophilic Inflammation in the pathogenesis of Chronic Obstructive Pulmonary Disease. COPD 2018;15:392-404.
8. Barnes PJ. Targeting cytokines to treat asthma and chronic obstructive pulmonary disease. Nat Rev Immunol 2018 Jul;18: 454-66.
9. George L, Brightling CE. Eosinophilic airway inflammation: role in asthma and chronic obstructive pulmonary disease. Ther Adv Chronic Dis 2016;7:34-51.
10. Saha S, Brightling CE. Eosinophilic airway inflammation in COPD. Int J Chron Obstruct Pulmon Dis 2006;1:39-47.
11. Singh D, Kolsum U, Brightling CE, et al. Eosinophilic inflammation in COPD: prevalence and clinical characteristics. Eur Respir J 2014;44:1697-700.
12. Tworek D, Antczak A. Eosinophilic COPD-a distinct disease phenotype. Adv Respir Med 2017;85:271-6.
13. David B, Bafadhel M, Koenderman L, et al. Eosinophilic inflammation in COPD: from an inflammatory marker to a treatable trait. Thorax 2021;76:188-95.
14. Siva R, Green RH, Brightling CE, et al. Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial. Eur Respir J 2007;29:906-13.
15. Yun JH, Lamb A, Chase R, et al. COPD Gene and ECLIPSE Investigators. Blood eosinophil counts thresholds and exacerbations in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2018;141:2037-47.e10.
16. Tashkin DP, Strange C. Inhaled corticosteroids for chronic obstructive pulmonary disease: what is their role in therapy? Int J Chron Obstruct Pulmon Dis 2018;13:2587-601.
17. Pascoe S, Barnes N, Brusselle G, et al. Blood eosinophils and treatment response with triple and dual combination therapy in chronic obstructive pulmonary disease: analysis of the IMPACT trial. Lancet Respir Med 2019;7:745-56.
18. Colak Y, Afzal S, Marott JL, et al. Type-2 inflammation and lung function decline in chronic airway disease in the general population. Thorax 2024;79:349-58.
19. Wagener AH, De Nijs SB, Lutter R, et al. External validation of blood eosinophils, FENO, and serum periostin as surrogates for sputum eosinophils in asthma. Thorax 2015;70:115-20.
20. Nakwan N, Thidarat Ruklerd T, Perkleang T, et al. The levels and correlations of FeNO, blood eosinophils and lung function in well-controlled asthma. Adv Respir Med 2022. doi: 10.5603/ARM.a2022.0015.
21. Brightling C, Greening N. Airway inflammation in COPD: progress to precision medicine. Eur Respir J 2019;54:1900651.
22. Oishi K, Matsunaga K, Shirai T, et al. Role of type2 inflammatory biomarkers in chronic obstructive pulmonary disease. J Clin Med 2020;9:2670.
23. Terry PD, Dhand R. The 2023 GOLD report: updated guidelines for inhaled pharmacological therapy in patients with stable COPD. Pulm Ther 2023;9:345-57.
24. Eltboli O, Mistry V, Barker B, et al. Relationship between blood and bronchial submucosal eosinophilia and reticular basement membrane thickening in chronic obstructive pulmonary disease. Respirology 2015;20:667-70.
25. Hastie AT, Martinez FJ, Curtis JL, et al. Association of sputum and blood eosinophil concentrations with clinical measures of COPD severity: an analysis of the SPIROMICS cohort. Lancet Respir Med 2017;5:956-67.
26. Kolsum U, Damera G, Pham TH, et al. Pulmonary inflammation in patients with chronic obstructive pulmonary disease with higher blood eosinophil counts. J Allergy Clin Immunol 2017;140:1181-4.e7.
27. Turato G, Semenzato U, Bazzan E, et al. Blood eosinophilia neither reflects tissue eosinophils nor worsens clinical outcomes in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2018;197:1216-9.
28. Kovalszki A, Weller PF. Eosinophilia. Prim Care 2016;43: 607-17.
29. Ragnoli B, Radaeli A, Pochetti P, et al. Fractional nitric oxide

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measurement in exhaled air (FeNO): perspectives in the management of respiratory diseases. Ther Adv Chronic Dis 2023;14:20406223231190480.
30. Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med 2011;184:602-15.
31. Louis R, Satia I, Ojanguren I, et al. European Respiratory Society Guidelines for the diagnosis of asthma in adults. Eur Respir J 2022;60:2101585.
32. Asthma: diagnosis, monitoring and chronic asthma management. London, UK: National Institute for Health and Care Excellence (NICE); 2021.
33. Lu Z, Huang W, Wang L, et al. Exhaled nitric oxide in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis. Int J Chron Obstruct Pulmon Dis 2018; 13:2695-705.
34. Karakioulaki M, Papakonstantinou E, Goulas A, Stolz D. The role of atopy in COPD and asthma. Front Med 2021;8:674742.
35. Donohue JF, Herje N, Crater G, et al. Characterization of airway inflammation in patients with COPD using fractional exhaled nitric oxide levels: a pilot study. Int J Chron Obstruct Pulmon Dis 2014;9:745-51.
36. Chen FJ, Huang XY, Liu YL, et al. Importance of fractional exhaled nitric oxide in the differentiation of asthma-COPD overlap syndrome, asthma, and COPD. Int J Chron Obstruct Pulmon Dis 2016;11:2385-90.
37. Schneider A, Brunn B, Hapfelmeier A, et al. Diagnostic accuracy of FeNO in asthma and predictive value for inhaled corticosteroid responsiveness: a prospective, multicentre study. EClinicalMedicine 2022;50:101533.
38. Lim CS, Rani FA, Tan LE. Response of exhaled nitric oxide to inhaled corticosteroids in patients with stable COPD: a systematic review and meta-analysis. Clin Respir J 2018;12:218-26.
39. Keeratichananont W, Kaenmuang P, Geater SL, et al. Correlation of fractional exhaled nitric oxide (FeNO) and clinical outcomes in patients with chronic obstructive pulmonary disease: A prospective cohort study. Respir Med 2024;229:107682.
40. Agustí A, Celli BR, Criner GJ, et al. Global Initiative for chronic obstructive lung disease 2023 report: GOLD executive summary. Eur Respir J 2023;61:2300239.
41. Graham BL, Steenbruggen I, Miller MR, et al. Standardisation of spirometry 2019 update. An official American Thoracic Society and European Respiratory Society technical statement. Am J Respir Crit Care Med 2019;200:e70-88.
42. Stanojevic S, Kaminsky DA, Miller MR, et al. ERS/ATS technical standard on interpretive strategies for routine lung function tests. Eur Respir J 2022;60:2101499.
43. Bowerman C, Bhakta NR, Brazzale D, et al. A race-neutral approach to the interpretation of lung function measurements. Am J Respir Crit Care Med 2023;207:768-74.
44. Daniel RA, Aggarwal P, Kalaivani M, et al. Prevalence of chronic obstructive pulmonary disease in India: a systematic review and meta-analysis. Lung India 2021;38:506-13.
45. Liu Y, Ma G, Mou Y, et al. The combined value of type-2 inflammatory markers in chronic obstructive pulmonary disease. J Clin Med 2022;11:2791.
46. Dhumal P, Singh V, Sarkar M, et al. Absolute blood eosinophil counts among 3,763 healthy, asthmatic, and COPD subjects across India. Eur Respir J 2023;62:3602.
47. Kiran CR, Kumari KA, Nair S, George T. Eosinophil count in COPD - do we need different cut-offs for our population? Pulmon 2021;23:151-6.
48. Christenson SA. COPD Phenotyping. Respir Care 2023;68: 871-80.
49. Rabe KF, Rennard S, Martinez FJ, et al. Targeting type 2 inflammation and epithelial alarmins in chronic obstructive pulmonary disease: a biologics outlook. Am J Respir Crit Care Med 2023;208:395-405.
50. Bhatt SP, Rabe KF, Hanania NA, et al. Dupilumab for COPD with type 2 inflammation indicated by eosinophil counts. N Engl J Med 2023;389:205-14.

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Informed consent: written informed consent was obtained from a legally authorised representative(s) to publish anonymised patient information in this article. The manuscript does not contain any person's data in any form.

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