Original Article

Role of Fractional Exhaled Nitric Oxide in Distinguishing Asthma-COPD Overlap Among Patients with COPD: A Cross-Sectional Study

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ABBRIVATIONS USED IN THIS ARTICLE

COPD = Chronic obstructive pulmonary disease

ACO = Asthma-COPD overlap

FeNO = Fractional exhaled nitric oxide

BMI = Body mass index

FEV_1 = Forced expiratory volume in first second

FVC = Forced vital capacity

GINA-GOLD = Global Initiative for Chronic Asthma and the Global Initiative for Obstructive Lung Disease

ppb = parts per billion

ICS = Inhaled corticosteroid

NOS = NO synthase

AUC = Area under the curve

IL = Interleukin

Abstract

Objective. Asthma-chronic obstructive pulmonary disease (COPD) overlap (ACO) and COPD share much of their clinical presentations, ensuring difficulties in their differentiation. As both the entities are characterised by airway inflammation, fractional exhaled nitric oxide (FeNO), as an inflammatory biomarker to distinguish between them, can be used for this purpose. The present study was done to assess the role of this local biomarker in distinguishing ACO among patients with COPD.

Methods. We enrolled 63 male stable patients of COPD from March 2019 to February 2020 from two tertiary care hospitals of Dhaka, Bangladesh. Among them, 51 patients were finally selected according to inclusion and exclusion criteria and divided into two study groups, ACO (n=26) and COPD-alone (n=25), according to GINA-GOLD Joint guidelines [Global Initiative for Asthma and the Global Initiative for Chronic Obstructive Lung Disease] on syndromic approach. The levels of FeNO were measured by NOBreath FeNO Monitor® (Bedfont, England).

Results. We observed that FeNO was significantly higher ($P<0.01$) in patients with ACO than COPD-alone. In addition, no statistically significant difference of FeNO was observed in patients with different stages of the disease severity in ACO and COPD-alone. Moreover, the correlation between FeNO levels and disease severity in both the groups was statistically insignificant. Area under the receiver operating characteristic curve of this biomarker was found as 0.724 and the optimal cut-off value was 29.5 parts per billion (ppb) to get the best diagnostic accuracy. At an optimal cut-off value, sensitivity, specificity were found to be 72.0% abd 84.6%, respectively. The positive predictive value and negative predictive value were 81.8%, 75.7%; whereas positive likelihood ratio, negative likelihood ratio and accuracy of FeNO were observed to be, 4.7%, 0.3%, 78.4%, respectively.

Conclusions. Fractional exhaled nitric oxide can play a substantial role in distinguishing ACO among the stable patients of COPD with good diagnostic accuracy. However, in our study no correlation of FeNO with the disease severity was obseved.

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Introduction

Chronic obstructive pulmonary disease (COPD) and bronchial asthma are frequently occurring airway diseases with chronic airway inflammation. Their incidence is increasing globally with an increase in the burden on health services.[1,2] In COPD, there is persistent airflow limitation, which is usually progressive.[1] However, in asthma, there is variable airflow limitation with wheeze, shortness of breath, chest tightness, cough that vary in intensity over time.[2]

These two distinct disease entities often overlap.[3] It has been reported that approximately one in four patients with COPD has asthmatic features.[4] On the other hand, patients with asthma may present with fixed airway obstruction over time.[5] In 2014, a new disease entity has been coined as 'asthma-COPD overlap (ACO)', which includes patients with several different phenotypes of airways disease caused by a range of different underlying mechanisms.[6]

As per treatment protocol proposed by Global Initiative for Asthma (GINA) and Global Initiative for Chronic Obstructive Lung Disease (GOLD) joint document, patients with ACO should be treated with inhaled corticosteroids (ICS).[6] In patients with COPD-alone, responsiveness of ICS is limited.[7] Moreover, it is also postulated that there is higher risk of subsequent pneumonia due to immunosuppressive characteristics of ICS.[8] Therefore, ICS should not be preferred in patients without an asthmatic component. For that reason, proper identification and clear differentiation between these two obstructive airway diseases is required.

In respiratory tract, nitric oxide (NO), a molecular gas, is produced in different types of cells, principally airway epithelial cells during the conversion of L-arginine to L-citrulline, catalysed by the NO synthase (NOS).[9,10] This enzyme has three distinct isoforms: neuronal (nNOS), endothelial (eNOS) and inducible (iNOS).[9] The isoforms nNOS and eNOS are synthesised at a constant rate, whereas iNOS is an inducible isoform. The activity of iNOS increases during certain inflammatory processes by different pro-inflammatory cytokines and bacterial endotoxins.[10] Thus, during airway inflammation in asthma[11], COPD[12], as well as in ACO[12], high amount of NO is produced in respiratory tract and is exhaled. This concentration of NO in exhaled air (FeNO) can be measured and used as an inflammatory biomarker of those obstructive airway diseases.[13]

To identify this newer respiratory phenotype among the patients with COPD, presently clinicians only rely on clinical and spirometric evaluation[6], both of which are relatively subjective methodologies. As both these obstructive airway diseases present with different intensities of airway inflammation, assessment of any local inflammatory biomarker, as FeNO, might aid in their proper identification, as well as staging of the severity for the initiation of appropriate treatment. Therefore, on the basis of this background, the present study was designed to evaluate the role of FeNO in distinguishing ACO among patients with COPD.

Material and Methods

This cross-sectional study was carried out in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU) from March 2019 to February 2020. Sixty-three male stable patients with COPD (age 40 to 80 years; body mass index (BMI) 18.6 to 24.9 Kg/m^2) were enrolled from the out-patient department of BSMMU and National Institute of Diseases of the Chest and Hospital (NIDCH), Dhaka, Bangladesh. All patients were diagnosed according to the GOLD criteria, *i.e.* post-bronchodilator forced expiratory volume in first second/forced vital capacity (FEV_1/FVC) <0.70.[7]

Among them, physician diagnosed patients with any other co-existent pulmonary disease, ischaemic heart disease, inflammatory bowel disease, inflammatory musculo-skeletal disorder, haematologic or endocrine disorder, malignancy, uncontrolled systemic hypertension, diabetes mellitus, dyslipidaemia, hepatic dysfunction or renal insufficiency were excluded. Moreover, patients with history of consuming systemic corticosteroids within four weeks prior to study, as well as if they were current smokers, were also excluded from the study. After exclusion, 51 patients were selected and informed written consent was taken from all patients. The protocol of this study involving the human subjects followed the ethical rule of Helsinki[14] and was approved by Institutional Review Board of the University.

All selected patients with COPD were divided into two study groups, ACO (n=26) and COPD-alone (n=25), according to syndromic approach for distinguishing ACO, from the GINA-GOLD joint document.[6] In addition, all the patients were categorised spirometrically for different stages of the severity of the disease, according to the GOLD criteria.[7]

The levels of FeNO were measured using the NOBreath FeNO Monitor® (Bedfont, England) according to the standard operating procedures recommended by the manufacturer using electrochemical sensor technique.[15]

Statistical Analysis

The data were expressed as mean with standard deviation (mean±SD), median with interquartile range and number with percentage. To compare the

qualitative data, Chi-square and Fisher's exact tests were done. In addition, to check the normal distribution of quantitative data, Shapiro-Wilk test was done. Then, independent sample 't' test was performed to compare normally distributed quantitative data between the two groups of patients. However, in case of the data showing skewed distribution, log transformation followed by Shapiro-Wilk test was performed. If the data is still in skewed distribution, Mann-Whitney U test was done between two groups of patients and Kruskal-Wallis test was performed to compare them between more than two groups.

Moreover, to find out any relationship between two variables, Spearman's rank correlation coefficient test was done. Furthermore, to determine area-under-the curve (AUC) and optimal cut-off values, receiver-operating-characteristics (ROC) curve analysis was performed. All statistical analysis was done using Statistical Package for the Social Sciences, USA (SPSS; Version 23) for Windows. A P value of <0.05 was considered to be significant.

Results

The general characteristics of the study patients are shown in table 1. No statistically significant difference was found between the two groups of patients with ACO and COPD-alone except patient frequency in stage IV.

In the present study, FeNO was significantly (P<0.01) higher in patients with ACO than that of patients with COPD-alone (Table 2). Among them, in patients without ICS, FeNO was significantly (P<0.01) higher in ACO than that of COPD-alone. However, in patients with ICS, no statistically significant difference of this biomarker between the two groups was observed (Table 2).

In the present study, no statistically significant difference of this biomarker was found in patients with different stages of the disease severity in ACO and COPD-alone (Figure 1). The correlation between FeNO levels and the disease severity in both the groups of patients was statistically insignificant (Figure 2).

The ROC curve analysis to diagnose ACO among patients with COPD, demonstrated that AUC was 0.724 (P<0.01; 95% CI 0.583–0.865) in overall (with or without ICS) COPD patients and 0.743 (P<0.01; 95% CI 0.585–0.902) in patients without ICS. To get the best diagnostic accuracy, the optimal cut-off values (according to highest Youden's index)[16] of FeNO were 29.5 and 19.5 ppb, in overall (with or without ICS) COPD patients and patients without ICS, respectively.

Table 1. General characteristics of the study patients (N=51)

Variables	Patients with ACO (n=26)	Patients with COPD-alone (n=25)	P value
Age (years) (Mean±SD)	56.9±10.6 (40-75)	61.6±9.8 (45-80)	0.103$^{\alpha}$
Weight (Kg) (Mean±SD)	60.4±4.8 (48-68)	57.4±5.8 (46-69)	0.051$^{\alpha}$
Height (cm) (Mean±SD)	168.0±8.1 (154-185)	165.0±6.3 (154-177)	0.276$^{\alpha}$
BMI (Kg/m^2) (Median IQR)	21.3 (20.1-22.9)	20.4 (19.2-22.4)	0.317$^{\beta}$
Smoking status			
Never smoker (N,%)	5 (19.2%)	2 (8.0%)	
Ex smoker (N, %)	21 (80.8%)	23 (92.0%)	0.419$^{\gamma}$
Pack-years (year) (Mean±SD)	25.38±12.58	29.13±8.74	0.254$^{\alpha}$
ICS use	7 (26.9%)	5 (20%)	0.743$^{\delta}$
Stages of severity			
Stage I	2 (7.7%)	1 (4.0%)	1.000$^{\gamma}$
Stage II	13 (50%)	7 (28.0%)	0.108$^{\delta}$
Stage III	10 (38.5%)	9 (36.0%)	0.896$^{\delta}$
Stage IV	1 (3.8%)	8 (32.0%)	0.011$^{\gamma}$

Statistical analysis was done by independent sample 't' test (α), Mann-Whitney U test (β), Fisher exact test (γ), Chi-Square test (δ).

Pack-year=(Number of cigarette smoked per day/20) X number of years smoked

Stage I=FEV_1>80%, Stage II=50%>FEV_1<80%, Stage III=30%>FEV_1<50%, Stage IV=FEV_1<30%7

*=statistically significant (P<0.05)

Table 2: Levels of FeNO in both the groups of study patients.

	Patients with		P value
	ACO (n=26)	COPD-alone (n=25)	
Overall	31.5 (18.5-51.0)	17.0 (13.0-26.5)	0.006
Without ICS	37.0 (20.0-51.0)	17.0 (13.0-25.5)	0.009
With ICS	29.0 (13.0-45.0)	21.0 (11.5-27.5)	0.291

Data are shown as median (interquartile range).

Statistical analysis was done by Mann-Whitney U test.

A P value of <0.01 is statistically significant

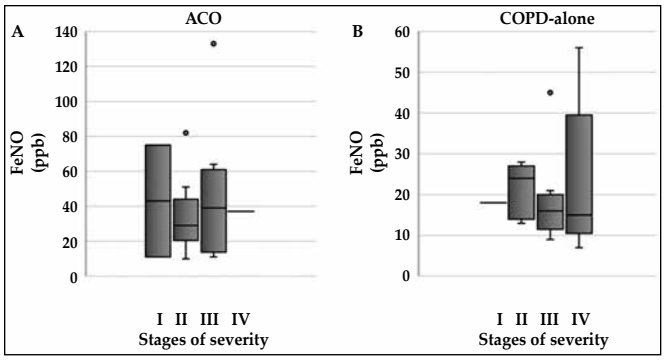

Figure 1. Median (interquartile range) of FeNO with different stages of disease severity in patients with (A) ACO and (B) COPD-alone. Statistical analysis was done by Kruskal-Wallis test.

Stage I=FEV_1>80%, Stage II=50%>FEV_1<80%, Stage III=30%>FEV_1<50%, Stage IV=FEV_1<30%[7]

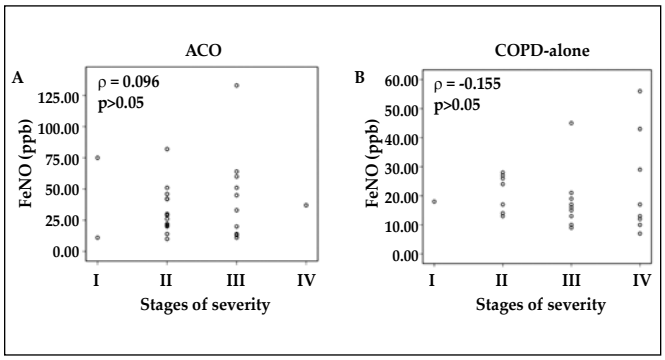

Figure 2. Correlation of FeNO with the disease severity in patients with (A) ACO and (B) COPD-alone.

Statistical analysis was done by Spearman's rank correlation coefficient test.

Stage I=FEV_1>80%, Stage II=50%>FEV_1<80%, Stage III=30%>FEV_1<50%, Stage IV=FEV_1<30%[7]

At 29.5ppb, the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio and accuracy of this biomarker were calculated as, 53.8%, 88.0%, 82.4%, 64.7%, 4.5, 0.5 and 70.6%, respectively in overall patients, at 19.5 ppb, the aforesaid indices were as, 79.0%, 70.0%, 71.4%, 77.8%, 2.6, 0.3 and 74.4%, respectively, in patients without ICS medication (Figure 3).

Discussion

In the present study, FeNO was significantly higher in ACO patients compared to that of COPD-alone, indicating its supportive role in distinguishing these two obstructive airway diseases. These results are in consonance with other studies performed globally.[17-19] It is well recognised now that patients with ACO shares features of both asthma and COPD, where there

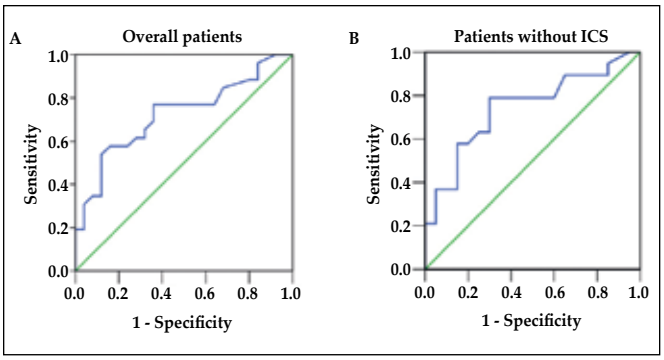

Figure 3. Curve of FeNO for distinguishing ACO among (A) overall (with or without ICS) COPD patients and (B) those without ICS.

is co-existence of type 2 helper T-cell (Th2) mediated inflammation along with neutrophilic invasion, releasing different Th2 inflammatory mediators.[20] Among them, interleukin-4 (IL-4), IL-13 robustly enhance inducible NO synthase (iNOS) activity and consequently increase the concentration of FeNO.[21,22] Moreover, this difference between these two groups of patients for this biomarker still remained statistically significant among ICS non-users, as compared to patients using ICS therapy. Results of the present study indicate limited usefulness of FeNO in distinguishing ACO among ICS user COPD patients. This hypothesis was supported by Takayama *et al.*[19] Being an anti-inflammatory agent, ICS might cause reduction of the airway inflammation, resulting in reduced production of NO in respiratory tract.[23] In addition, as ACO is more responsive to ICS therapy than COPD-alone[24], it might cause more decrement of FeNO in ACO, explaining no significant difference of this biomarker between the two groups of our patients, who were on ICS medication.

Results of the present study showed no significant difference of FeNO levels with different stages of severity in both the group, which is similar to that described in other studies.[12,17] Also, there was no statistically significant correlation between FeNO and disease severity in both groups of the present study. Therefore, we postulate that this biomarker level is not related to severity of these obstructive airway diseases. These observations might claim low utility of FeNO in predicting severity of ACO, as well as COPD-alone. However, as all our study patients were in stable state, there might be no difference in inflammatory, intensity and hence, NO production in their airways.

In our study, ROC curve analysis showed good diagnostic accuracy (AUC 0.724) of FeNO in distinguishing ACO among overall COPD with or without ICS medication. Similar observations were reported by others.[17,18,25]

The specificity and positive predictive value were found >80% in overall COPD patients, with or without ICS medication at an optimal cut-off value of 29.5ppb of this biomarker, thereby, indicating less probability of being false positive results in distinguishing ACO among them. This might reduce the inappropriate treatment with ICS in COPD-alone patients.

In our study, at optimal cut-off values, this biomarker showed positive likelihood ratio and negative likelihood ratio within a range of 2 to 5 and 0.2 to almost 0.5, respectively, in both overall (with or without ICS medication) and ICS non-user patients.

Conclusions

It is postulated that FeNO may be a good marker in distinguishing ACO from patients with COPD-alone, though it possesses no role in the disease severity assessment. More studies are required to further confirm these preliminary observations.

There were some limitations of our study. As allergic rhinitis, eosinophilia and use of ICS medication can affect FeNO level, we intended to exclude stable patients of COPD with these criteria. But this was not possible due to the small sample size of our study. To explore the role of inflammatory biomarkers for distinguishing ACO among the patients with COPD even in exacerbation state, further studies are needed.

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