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Comparison of Fractional Exhaled Nitric Oxide in Elderly Patients with Asthma-chronic Obstructive Pulmonary Disease Overlap and Other Airway Inflammatory Diseases

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ABSTRACT

The exact role of fractional exhaled nitric oxide (FeNO) in older patients with chronic inflammatory diseases including asthma-chronic obstructive pulmonary disease (COPD) overlap (ACO) remains unclear. This study aimed to investigate the differences in FeNO levels of elderly patients with ACO, asthma, COPD, and chronic cough.

We conducted a retrospective study analysing the data of stable outpatients from Pulmonary Department of the Second Clinical College, Jinan University. All participants (Age≥55 years) were divided into the ACO group (n=19), asthma group (n=16), COPD group (n=25), and chronic cough group (n=22). The clinical data such as peripheral eosinophil counts, serum high sensitivity C-reactive protein (hs-CRP), FeNO, and spirometry was collected, and the correlations between FeNO levels and systemic markers or spirometric indices were analyzed.

Patients with ACO and asthma had significantly elevated FeNO levels (37.7±16.5, and 36.3±17.7 ppb) compared with COPD, and chronic cough patients (21.9±10.3, and 16.1±8.8 ppb). The FeNO levels were negatively associated with forced expiratory volume in 1 second **(**FEV1, p=0.003), FEV1% predicted (p=0.012), and FEV1/forced vital capacity **(**FVC, p=0.002) in all groups. However, there were no significant correlation between FeNO levels and FVC, peripheral eosinophil counts, or serum hs-CRP (p>0.05).

Elderly patients with ACO have higher levels of FeNO, when compared with patients with COPD or chronic cough. These findings suggest that FeNO measurement may provide an important implication for the etiological diagnosis of ACO in the elderly patients.

Keywords: Asthma-chronic obstructive pulmonary disease overlap; Asthma; Elderly; Nitric oxide

INTRODUCTION

Adult-onset asthma and chronic obstructive pulmonary disease (COPD) are major public health problems in the general population, because they are associated with high prevalence, increased morbidity

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and healthcare costs.[1,2] With ageing, there is an increasing combination of symptoms of asthma and COPD. This condition has been labelled "Asthma COPD Overlap (ACO)", which is defined as having both airway hyperreactivity (AHR) and incompletely reversible airway obstruction.[3,4] More rapid disease progression and worse disease-related quality of life are reported to be associated with ACO compared with COPD or asthma alone.[5,6] Therefore, it's important for physicians to find the proper tests in identifying and managing lung diseases in the elderly such as ACO, asthma, COPD, and chronic cough.

Airway inflammation is now recognized as a central process in the pathogenesis of such chronic lung diseases.[7,8] However, it can be quite difficult to determine and identify the type of airway inflammation in the older patients. Fractional exhaled nitric oxide (FeNO) was proposed as a non-invasive biomarker for airway inflammation. The availability of nitric oxide (NO) analyzers made the measurement of FeNO a rapid and inexpensive tool to monitor Th2-mediated airway inflammation. While there have been a number of studies on measuring FeNO in both asthma and COPD,[9,10] the exact role of FeNO in older patients with chronic inflammatory diseases including ACO remains unclear. The aim of our study was conducted to clarify the characteristics of FeNO levels in elderly patients with ACO or other airway inflammatory diseases, and investigate its relationship with systemic inflammation, and pulmonary function. These findings may suggest an important role of FeNO in the clinical diagnostic practice in elderly patients with ACO.

MATERIALS AND METHODS

This study was conducted by retrospectively scanning the files of 136 stable outpatients (Age≥55 years) with ACO, COPD, asthma, or chronic cough who performed both spirometry and FeNO measurement at the Pulmonary Department, at the Second Clinical College, Jinan University, Shenzhen, China, from October 1, 2015 to Mar 31, 2016. A total of 82 patients who met the study criteria were enrolled for the study. This project was approved by the Ethics Committee of the Second Clinical College of Jinan University, Shenzhen, China, which waived the requirement for informed consent.

Baseline clinical characteristics including gender, age, body mass index (BMI), smoking status, and laboratory data were obtained from each participant. Spirometry and FeNO levels were obtained in all subjects. The correlation among these lung function parameters and FeNO levels in different groups were calculated.

Study Population

Diagnostic classification was performed using study inclusion criteria (Table 1): 1) The ACO group was comprised of subjects with respiratory symptoms, incompletely reversible airflow obstruction (post bronchodilator forced expiratory volume in 1 s/forced vital capacity [FEV1/FVC]<70%, and post bronchodilator FVC<80% of predicted), as well as airway hyper-responsiveness (AHR) or bronchodilator hyper-responsiveness (BHR); 2) the asthma group was defined by the GINA strategy document (post bronchodilator FEV1/FVC≥70%, and post bronchodilator FEV1≥80% of predicted, and AHR or BHR); 3) the COPD group met the COPD diagnostic criteria of the 2011 GOLD guidelines (post bronchodilator forced expiratory volume in 1 s/forced vital capacity [FEV1/FVC]<70%, and post bronchodilator FVC<80% of predicted, and no AHR or BHR); 4) the chronic cough group was made up with subjects with chronic cough, and both no airflow obstruction (post bronchodilator FEV1/FVC≥70%, and post bronchodilator FEV1≥80% of predicted) and no AHR or BHR. Study exclusion criteria included the following: 1) cases who were suffering from respiratory failure, pneumothorax, fractured rib(s), tuberculosis, severe cardiovascular disease, liver disease, renal dysfunction, or malignant tumour; 2) use of bronchodilator and/or oral steroids within the last 2 days; 3) cases with a history of infection within the last 3 weeks; 4) cases who were current smokers or had ceased within the previous 12 months.

FeNO Measurements

Before spirometry analyses, all subjects underwent standard measurement of FeNO at an exhalation flow rate of 0.05 L/s, during single exhalation, using the NIOX MINO (Aerocrine AB, Solna, Sweden), according to the manufacturer's instructions. The measurement unit was expressed as ppb. The FeNO measurements were consistent with the American Thoracic Society (ATS) / European Respiratory Society (ERS) 2005 guidelines methods.[11] All patients refrained from eating and drinking for at least 1 hour prior to the

Table 1. Physiological characteristics and definition of study groups (ACO, asthma, COPD, chronic cough)

	ACO Group	Asthma Group	COPD Group	Chronic cough Group
Respiratory symptoms	+	+	+	+
Post bronchodilator FEV1/FVC%	<70%	≥70%	<70%	≥70%
Post bronchodilator FEV1% predicted	<80%	≥80%	<80%	≥80%
AHR or BDR	+	+	-	-

ACO, asthma-chronic obstructive pulmonary disease overlap; COPD,chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; AHR, Airway Hyperresponsiveness (provocative dose of histamine causing a 20% fall in FEV1 [PC20] < 8 mg/mL of histamine); BDR, Bronchodilator responsiveness (post-bronchodilator FEV1 increased ≥200 mL and 12% compared with pre-bronchodilator FEV1).

FeNO measurement. Each patient was allowed up to no more than 6 attempts to perform a single-exhalation maneuver, and the FeNO value was calculated as the mean of 3 correct exhalations. Following ATS/ERS 2005 guidelines, the normal exhaled NO value was set as 5–35 part per billion (ppb) for healthy adults.

Spirometry Measurements

Subsequent to the FeNO measurements, patient spirometry testing was performed using a V6200 Series Autobox (SensorMedics, USA) spirometer device. Lung function test parameters like FEV1, FVC, FEV1/FVC%, and FEV1/FVC were collected according to the methods of the ATS/ ERS task force.[12] A predicted ratio for each parameter was calculated based on age, sex, height, and race.

Peripheral Eosinophil Counts and Serum High Sensitivity CRP Levels

Peripheral eosinophil counts were determined via complete blood count test (Sysmex XN-3000, Japan), using impedance and optic scatter method. Serum high sensitivity CRP (hs-CRP, Roche Cobas C501, Germany) levels were measured using an immunoturbidimetric assay according to the manufacturers' instructions.

Statistical Evaluation

SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical evaluations. Data was expressed as mean±standard deviation, or case numbers/total patients (%). Analysis was performed using the One-way ANOVA test for comparison of more than 2 groups, as appropriate. The two-sample Wilcoxon Rank Sum test was used to test non-parametric data between 2 independent groups, and the Chi-Square test for categorical data. In order to identify the correlation between FeNO and FVC, FEV1, FEV1% predicted, or FEV1/FVC%, variables were analysed using the Pearson correlation coefficients. $p<0.05$ was accepted as a statistically significant difference.

RESULTS

Baseline Characteristics of the Study Populations

Patient demographic information is summarized in Table 2. Of all patients in the per protocol population, 19 (23.2%) were admitted to the ACO group; 16 (19.5%) to the Asthma group; 25 (30.5%) to the COPD group, and 22 (26.8%) to the chronic cough group. The mean age was 64.6 years (range 55–78 years) and 73.2% were men. There was no difference in anthropometric parameters age, gender and BMI among the groups. Among the 19 patients with ACO, men were 13 (68.4%) of all patients and the median age was 64.8 years. There were more smokers in the ACO group (57.9%) than in the Asthma group (25.0%, $p=0.037$). The eosinophil counts (245.8/mm^3) of ACO group were higher than that of COPD group (120.8/mm^3, $p<0.001$), and there was no difference in serum hs-CRP levels within the four groups ($p=0.264$). Furthermore, there was a significant decrease in lung function parameters including FVC (2.50±0.50L), FEV1 (1.39±0.35L), FEV1% predicted (58.81±9.41%), and FEV1/FVC ratio (55.39±6.28%) compared to the Asthma ($p<0.001$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively), and chronic cough ($p=0.002$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively) groups. Other details in the Asthma, COPD, and chronic cough groups are shown in Table 2.

Comparison of FeNO Levels between Groups

The FeNO levels in different groups are shown in Table 3. The FeNO levels of ACO (37.7±16.5 ppb) and Asthma group (36.3±17.7 ppb) were higher in the COPD (21.9±10.3 ppb), and chronic cough (16.1±8.8ppb) group, while there was no significant difference between ACO and asthma groups (p=0.833). All subjects in ACO group had FeNO levels of >20 ppb, except for two patients; these patients had FeNO level of 15 and 18 ppb. 10 (52.6%) of total 19 in ACO group had FeNO levels of >35 ppb. Furthermore, the proportion of subjects with an elevated FeNO level also had significant differences among the 4 groups (p<0.001).

Correlation Analysis of FeNO in the Study Groups

Overall, the FeNO level in all patients was negatively associated with FEV1 (r=-0.280, p=0.003), FEV1% predicted (r=-0.241, p=0.012), and FEV1/FVC (r=-0.290, p=0.002) in the study groups (Figure 1A-1C). However, the FeNO levels have no association with FVC (r=-0.188, p=0.053) (Figure 1D), eosinophil counts (r=0.263, p=0.215), and hs-CRP (r=-0.021, p=0.923).

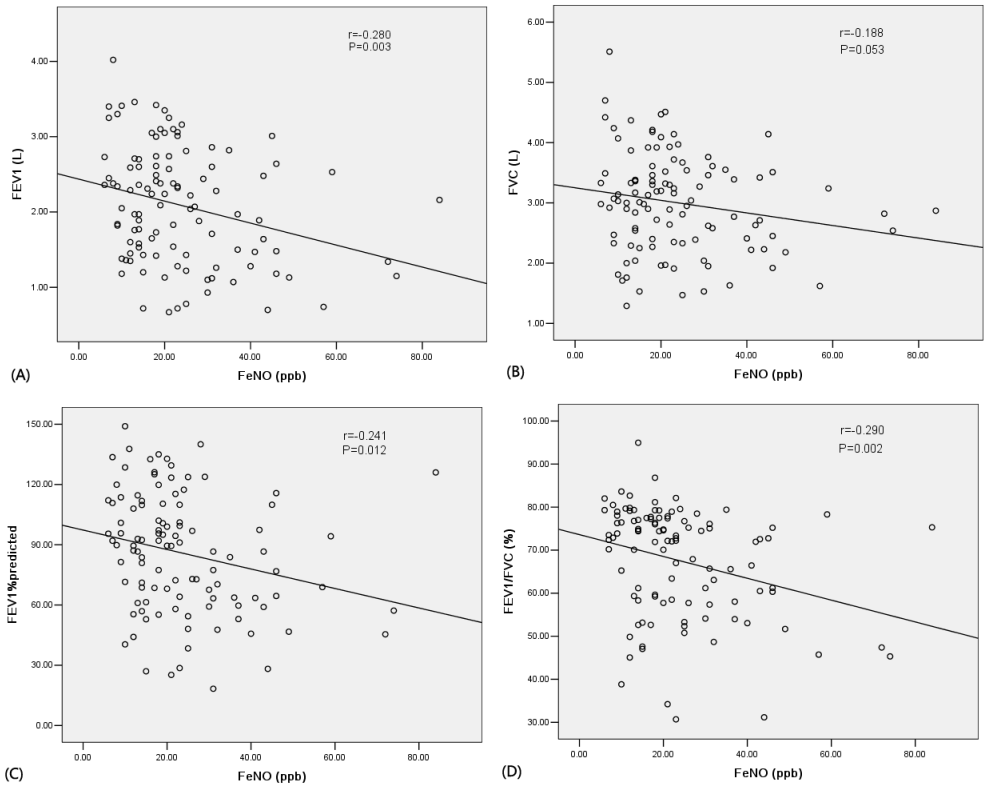

Figure 1. Correlation analysis of fractional exhaled nitric oxide (FeNO) in all four groups (asthma--chronic obstructive pulmonary disease [COPD] overlap group, asthma group, COPD group, and chronic cough group). (A) Correlation between FeNO with forced expiratory volume in 1 second (FEV1) in all four groups, (B) Correlation between FeNO with forced vital capacity (FVC) in all four groups, (C) Correlation between FeNO with FEV1% predicted in all four groups, (D) Correlation between FeNO with FEV1%/FVC in all four groups.

Table 2. Clinical characteristics of study groups (ACO, asthma, COPD , chronic cough)

Parameter	ACO Group (n=19)	Asthma Group (n=16)	COPD Group (n=25)	Chronic cough Group (n=22)	*p* value
Male, number (%)	13(68.4)	11(68.8)	21(84.0)	15(68.2)	0.555
Age, years, mean (SD)	64.8(6.5)	62.2(5.9)	65.4(7.0)	65.3(6.9)	0.444
BMI, mean (SD)	22.9(1.9)	24.0(2.0)	23.0(3.7)	23.6(3.6)	0.685
Smokers, ex (%)	11(57.9)	4(25.0)※, †	21(84.0)*	13(59.1)*	0.002
EOS counts, /mm^3, mean (SD)	245.8(145.2) ※	182.5(97.4)	120.8(97.9) †	180.2(117.3) ※	0.004
Hs-CRP, mg/L, mean (SD)	4.67(2.63)	3.64(2.78)	3.95(2.95)	2.98(2.47)	0.264
FVC, L, mean (SD)	2.50(0.50)*, †	3.49(0.51)※	2.57(0.76)*, †	3.23(0.72)※	<0.001
FEV1, L/s, mean (SD)	1.39(0.35)*, †	2.59(0.37)※	1.47(0.56)*, †	2.44(0.52)※	<0.001
FEV1% predicted, mean (SD)	58.82(9.41)*, †	97.44(24.40)※	56.58(16.75)*, †	100.75(17.04)※	<0.001
FEV1/FVC%, mean (SD)	55.39(6.28)*, †	74.37(2.25)*, †	53.65(10.93)*, †	75.77(3.46)※	<0.001

*p<0.05 vs Asthma; ※p<0.05 vs COPD; †p<0.05 vs Chronic cough.

SD, standard deviation; ACO, asthma-chronic obstructive pulmonary disease overlap; COPD,chronic obstructive pulmonary disease; BMI, Body mass index; EOS, eosinophil; hs-CRP, high sensitivity C-reactive protein; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

Table 3. Levels of FeNO in study groups (ACO, asthma, COPD , chronic cough)

	ACO Group (n=19)	Asthma Group (n=16)	COPD Group (n=25)	Chronic cough Group (n=22)	*p* value
FeNO (ppb)					
mean (SD)	37.7(16.5)※, †	36.3(17.7)※, †	21.9(10.3)*	16.1(8.8)*	<0.001
FeNO>35ppb, n (%)	10(52.6)※, †	9(56.3)※, †	3 (12.0)*	1(4.5)*	<0.001

*p<0.05 vs Asthma; ※p<0.05 vs COPD; †p<0.05 vs Chronic cough.

Abbreviations: SD, standard deviation; ACO, asthma-chronic obstructive pulmonary disease overlap; COPD,chronic obstructive pulmonary disease; FeNO, fractional exhaled nitric oxide; ppb, Parts per billion.

DISCUSSION

The results from our study suggest that increase in FeNO do occur in elderly patients with ACO or asthma. Although the FeNO levels in COPD group were slightly higher than the chronic cough group, there was no significant difference in the two groups. The elderly patients with chronic airway inflammatory diseases had negative relationship between some lung function parameters and FeNO levels.

Based on the results from previous studies, the measurement of FeNO levels has recently been investigated as a complementary tool to assess the aetiology of patients with wheeze or chronic cough.[13,14] Even though the increase in FeNO levels has shown most valuable to rule in patients with eosinophilic airway inflammatory pattern,[15,16] it also displays the tone of assessing airway remodelling and responsiveness.[14,17,18] However, the diagnostic accuracy of FeNO detecting chronic airway inflammatory diseases in the elderly hasn't been confirmed. In our study, FeNO levels were substantially higher in patients with ACO/asthma compared with COPD, and chronic cough patients. Considering information on FeNO levels being affected by eosinophilic and non-eosinophilic inflammation,[19,20] the ACO group may be identified by the features that it shares inflammations of both asthma and COPD, and more tend to the eosinophilic phenotype. Thus, FeNO measurement might be a possible screening method to differentiate ACO with COPD and other non-eosinophilic airway

diseases.

In a study by Schneider A et al[10] the exhaled NO level was found to be higher (44.3 ppb) in asthmatic patients with eosinophilic inflammation, and lower (18.5 ppb) with neutrophilic inflammation. We found the median level of FeNO in the Asthma group (36.3 ppb) was slightly under the previously value with eosinophilic inflammation, which might be due to the genetic, regional difference, and small sample size in our study. On the other hand, non-eosinophilic inflammation was likely to contribute to this low FeNO level,[21] suggesting that FeNO measurements should be used to identify the inflammatory phenotypes of asthma in the elderly, and to help tailor asthma therapy appropriately.[22,23] Moreover, the median level of FeNO in the elderly with COPD or chronic cough was below the previously published threshold levels of adult healthy subjects (26 ppb).[24] Based on this it can be concluded that the ability of FeNO to estimate the inflammatory properties of COPD or chronic cough is limited, and other factors including smoking status, oxidative products and atopic symptoms should be always considered or analyzed.

We demonstrated direct relationship between FeNO, lung function, and inflammatory markers in the elderly with ACO or other airway inflammatory diseases. The observation that has received little attention elsewhere. In recent years, many studies have demonstrated hs-CRP is an acute phase proteins used as a surrogate marker for systemic inflammation in airway. Eosinophils while having a role as phagocytes, also appear to function in a broad spectrum of allergic inflammation and physiologic immune responses. We choose to determine the two inflammatory markers because both have been commonly studied in some airway inflammatory studies. Our study showed that the FeNO levels had no correlation with serum hs-CRP, peripheral eosinophil counts, but the negative associations between FeNO and FEV1, predicted FEV1%, or FEV1/FVC (p=0.003, 0.012, and 0.002, respectively) were presented in all the subjects. These results were because FEV1 measures airway calibre[25] and reflects the airway responsiveness. In the study conducted on well-controlled asthmatics (mean age 61), strong negative correlation was also identified between FEV1/FVC and FeNO levels.[26] While in the study conducted on healthy children, Zhang et al demonstrated that FeNO has no correlation with spirometry measurements.[27] This can be explained by the differences in the studied population. An alternative explanation for the discrepancy between the current findings and previous studies might be that some potential risk factors, such as ages, gender, metabolism and immunity, could not be balanced in different patients, which might affect the power of the study with respect to these factors. However, the correlation coefficient between predicted FVC and FeNO levels was only 0.053. A larger sample size would be required to obtain a statistical significance with this correlation coefficient.

There were several limitations to our study. These results were elucidated from retrospective analysis and we could not thoroughly deal with other factors that affect NO productions, such as induced sputum eosinophil counts. Furthermore, we simply focused the initial FeNO levels, and didn't follow-up serial FeNO levels regularly. It would be of interest to evaluate the role of FeNO monitoring the treatments of older patients with ACO.

In conclusions, our study demonstrated that increases in FeNO levels could be observed in elderly patients with ACO or asthma, while FeNO values of COPD and chronic cough patients were intermediate or low. There was no significant difference between COPD and chronic cough patients. Measurement of FeNO provides useful information to evaluate the airway calibre and responsiveness, and may indicate the management of elderly patients with ACO, asthma, COPD, or chronic cough. The combination of FeNO levels and spirometric parameters might have a potentially useful role for the etiological diagnosis of airway inflammatory diseases in the elderly. However, further large-scaled, prospective studies needed to expand the clinical application of measuring FeNO to elderly population with ACO.

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