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Diagnostic value of fractional exhaled nitric oxide for asthma-chronic obstructive pulmonary disease overlap syndrome

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Abstract

To examine the difference in the fractional exhaled nitric oxide (FeNO) between chronic obstructive pulmonary disease (COPD) patients with asthma-COPD overlap syndrome (ACOS) and patients with Non-ACOS COPD (Non-ACOS) and to investigate the correlation between FeNO levels and the differential cell counts of eosinophils in induced sputum, in order to explore the diagnostic value of FeNO in ACOS.

A prospective, case-control study was performed on 53 cases of ACOS group and 53 cases of Non-ACOS group in the Respiratory Medicine Outpatient of Zhangzhou Municipal TCM Hospital, Affiliated to Fujian University of Traditional Chinese Medicine. The FeNO levels and induced sputum cell counts were determined and the correlation between FeNO levels and eosinophile percentage was analyzed by *Pearson* linear correlation analysis.

The FeNO levels in patients with ACOS (37[24.5–53.0]) ppb were significantly higher than those of patients with Non-ACOS (20 [15.5–24.5] ppb) ($P < .01$). Also, the percentage of eosinophils in induced sputum in the ACOS group (5.70 [1.50–17.62]%) were significantly higher than those of the Non-ACOS group (0.50 [0.00–1.00]%) ($P < .01$). FeNO in both groups correlated positively with the percentage of eosinophils in induced sputum ($P < .01$), with a correlation coefficient r of 0.521. The area under the receiver operating curve of FeNO for the diagnosis of ACOS phenotype was 0.815 ($P < .01$), the sensitivity and specificity reach highest when the cut off value was 25.50 ppb.

The FeNO in patients from the ACOS group were significantly higher than those in Non-ACOS group and were moderately correlated with the percentage of eosinophils in induced sputum. The results indicated that FeNO may be used as a diagnostic index for ACOS, in addition to the induced sputum.

Keywords: asthma-COPD overlap syndrome, fractional exhaled nitric oxide, induced sputum, sputum eosinophils

1. Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by persistent airflow limitation, which is related to inhalation of tobacco smoke and other harmful gases and pollutants. Evidence suggests there is heterogeneity in COPD, and patients with the same degree of pulmonary function can be significantly different in clinical symptoms and imaging manifestations.[1] Patients with similar symptoms or risk factors may have significant differences in response to the same drug.[2] In 2010, Han et al[3] first proposed the concept of COPD phenotype, according to the symptoms, acute exacerbation, effectiveness of treatment, degree of progression, and death related to the same characteristics of patients with COPD. In 2012, Spanish Asthma-COPD overlap syndrome (ACOS) diagnostic consensus (referred to as the 2012 Spanish ACOS Diagnostic Consensus)[4] proposed phenotypic diagnosis, and within this context ACOS was distinguished as an independent phenotype as well as others. In 2014, Global Prevention and Control Initiative for Asthma (GINA) issued guidelines concerning ACOS.[5] According to epidemiological survey: ACOS accounts for 10% to 20% of COPD.[6] In 50 to 59-year-old patients, the prevalence rate is about 23%, and in 70 to 79 year old patients this rate increases to 52%[7] with over 50% of all obstructive lung disease occurring in elderly patients.[8] These patients have frequent acute exacerbations of respiratory symptoms, a poorer quality of life, and increased mortality rate.[9,10]

Research shows ACOS has both chronic obstructive pulmonary and asthma airway inflammation characteristics, accompanied by increased eosinophils and neutrophils.[11] Eosinophilic airway inflammation is associated with the onset of asthma.[12] Exhaled fractional exhaled nitric oxide (FeNO) as a hot spot in airway inflammation research, has advantages of more non-invasive, simple, fast, repeatability, the patient has little pain compared with traditional induced sputum examination. The 2012 Spanish ACOS Diagnostic Consensus also proposed that elevated sputum eosinophils could be used as main criteria for ACOS diagnosis, while in 2014, GINA did not provide clear

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diagnostic criteria. The diagnostic criteria for ACOS are still controversial, and the diagnostic value of FeNO for ACOS has not been discussed. Studies show that the level of FeNO can better assess airway inflammation in asthmatic patients, including children and adults.[13,14] Karampitsakos and Gourgoulianis[15] believes that FeNO can help identify asthma and ACOS phenotype. Up to now, the research on diagnostic value of FeNO in COPD is relatively less, this study was designed to investigate the level of FeNO in patients with ACOS phenotype and its correlation with the differential of eosinophils in induced sputum, to investigate the diagnostic value of FeNO for patients with ACOS phenotype.

2. Patients and methods

2.1. Study design

2.1.1. Subjects. A total of 106 patients with stable COPD were enrolled in this study between May 2015 and May 2016, at the Respiratory Medicine Outpatient of Zhangzhou Municipal TCM Hospital, affiliated to Fujian University of Traditional Chinese Medicine. The study was approved by the Ethical Research Committee of Zhangzhou Municipal TCM Hospital, Affiliated Hospital of Fujian University of Traditional Chinese Medicine (No.2014–004). All subjects signed informed consents. Patients were divided into an ACOS group and a COPD group, according to the following criteria.

2.1.2. Diagnostic criteria. Diagnostic criteria of COPD: As per the Diagnosis and Treatment of COPD (2013 Revision) report, developed by the COPD Group of Respiratory Diseases Branch, Chinese Medical Association,[16] COPD was diagnosed a $FEV_1/FVC\% < 70\%$, after inhalation of a bronchodilator and continuous limitation of air flow, after exclusion of other diseases.

Diagnostic criteria of ACOS: In accordance with the 2012 Consensus document on the overlap phenotype COPD-asthma in COPD,[4] the diagnosis of ACOS is established by primary criteria: strong positive bronchial dilation test (BDT) in patients with COPD (FEV_1 improved >400 mL, improvement rate >15%); increased sputum eosinophilia or asthma diagnosis. Secondary criteria: COPD patients with elevated total serum IgE; history of allergies; positive BDTs (FEV_1 improved >200 mL, improvement rate >12%) on ≥2 occasions. The ACOS diagnosis requires the meeting of 2 primary criteria or 1 primary and 2 secondary criteria.

Diagnostic criteria of other COPD phenotype included other phenotypes that met the COPD criteria, but failed to meet ACOS criteria.

Exclusion criteria: upper respiratory tract infection in the recent 2 weeks; COPD patients in acute onset phase; use of systemic glucocorticoid in the recent month; diagnoses of bronchiectasis, tuberculosis, cystic fibrosis, diffuse panbronchiolitis, bronchiolitis obliterans, despite airflow obstruction; underlying serious cardiovascular, liver, kidney, or hematopoietic disease; refusal to cooperate or inability to communicate.

2.2. Methods

2.2.1. Pulmonary function test. Pulmonary function and bronchodilation tests were performed using a pulmonary function equipment (JAEGER, Germany). The lung function tests were completed by professional staff at the Lung Function Test Laboratory of Zhangzhou Municipal TCM Hospital, Affiliated Hospital of Fujian University of Traditional Chinese Medicine. The tests were repeated 3 times for each person, with best value for FEV_1 and FEV_1 percentage among predicted value ($FEV_1\%$) being recorded.

2.2.2. FeNO measurement. FeNO was measured by a method recommended by the American Thoracic Society/European Respiratory Society (ATS/ESR) Committee,[17] using a Nano Coulomb Breath Analyzer (model: Sunvous-P100, Sunvou Medical Electronics CO., Ltd.). There was no flowers, obvious dust, or peculiar smells from furniture, wall paint, and other ornaments indoors; 2 hours before testing and during the test period, smoking was prohibited indoors; the indoor temperature was maintained at 20 to 28 °C and relative humidity at 20% to 60%. Subjects were fasted within 1 hour before the test and they were not allowed to drink coffee, tea, carbonic acid, and soymilk drinks or super cooled and overheated drinking water, no vigorous exercises, no active or passive smoking. During testing, subjects sit or stood straight, and their heads kept natural level; and when the filter was placed in the mouth, the tongue could not plug the filter mouth to ensure no leak from the mouth, and loosened the too tight belt, chest strap, and clothes, etc.

2.2.3. Measurement methods. Firstly, subjects had calm breathing at least 3 cycles, and at the last exhalation, the gas in the lungs should be emptied; secondly, the filter was placed in the mouth, at this time, exhalation was not allowed and if exhalation happened, the instrument would automatically suspend, then the above steps first should be repeated; thirdly, when the exhalation speed was $<50\,mL \pm 10\%/s$, the subjects would be guided to adjust the intensity of exhalation, if the exhalation speed was not within the required range for >1.2 seconds accumulatively, the instrument would automatically pause, to repeat the above steps first and second, if the exhalation speed failed to meet the test requirements for 6 consecutive times, it would be included in test failure; fourthly, if alarm of instrument occurred during the test process due to environment factors such as strong cell phone signal or electromagnetic interference, then the above steps first, second, and third should be repeated; this case would not be included in the poor test compliance or test failure. The test results were expressed in ppb (parts per billion).

2.2.4. Sputum induction and differential cell counts in induced sputum. Referring to our laboratory the previous research methods[12]: after inhaling 200 μg salbutamol for 15 minutes, subjects inhaled 3.5% hypertonic saline by ultrasonic atomization for another 15 minutes, at time intervals of 5 minutes. At any time, when patients felt sputum, they were asked to blow their nose and gargle following deep cough. Their sputum were collected in a graduated sterile dry cup and sent to the laboratory for treatment (or kept at 4 °C for no more than 2 hours). For counting cells in induced sputum cells, an equal volume of phosphate buffer (PBS) containing 0.1% dithiothreitol (DTT) were added to the sputum samples and vortexed. The volume of completely liquefied sputum was recorded, centrifuged at 1500r/min for 10 minutes, and the supernatant were collected for testing. The pellet were resuspended in 50 mL PBS solution, filtered through a 48 μm sieve, re-centrifugated, and the supernatant were discarded and 1 mL of sediment were used for tests. Cell viabilities were assessed by trypan blue staining and samples with over 50% surviving cells were considered viable. The numbers of inflammatory and squamous epithelium cells were counted with a Neubauer hemocytometer (Neubauer Precicolor HBG, Germany). Cell smears were produced by a

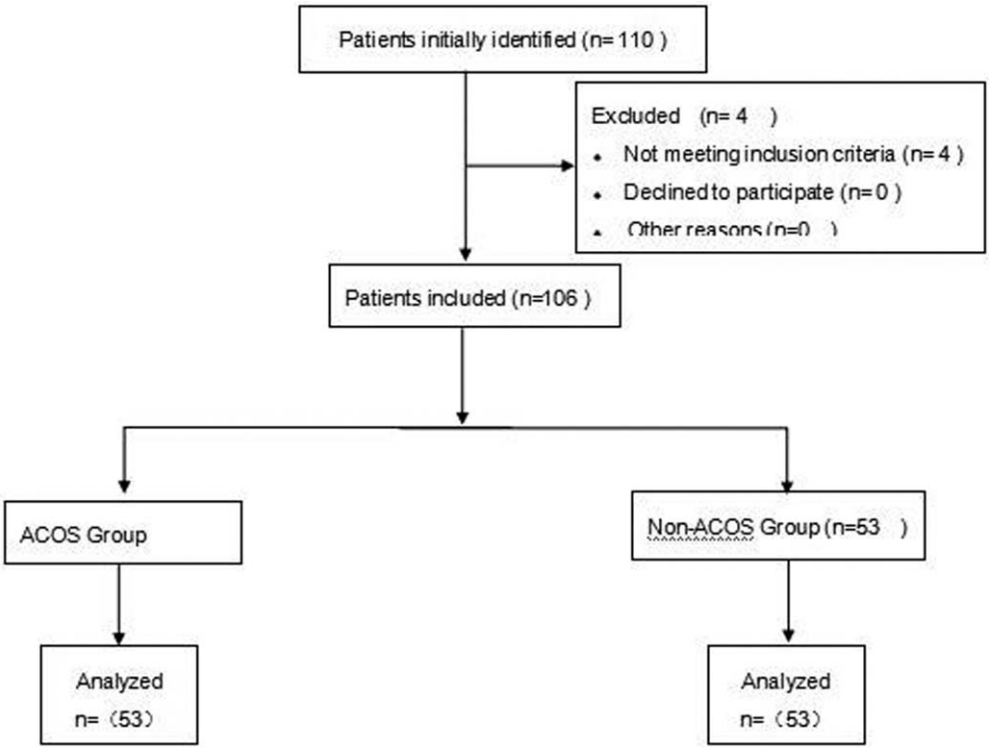

Figure 1. Flow chart of enrollment of patients and completion of the study.

smear centrifuge and analyzed by using Wright staining. Four hundred non-squamous epithelium cells were counted under the microscope (Olympus CX31, Japan) with an oil immersion lens.

Pulmonary medication at baseline included inhaled steroid and bronchodilator according to the 2015 Global strategy for the diagnosis, management, and prevention of COPD.[18]

2.3. Statistical analysis

All statistical analyses were performed using the SPSS 20.0 statistical software (SPSS Corp, Los Angeles). For the normality test, data were expressed as $\overline{X} \pm S$. The 2 sample groups were compared using an independent *t* test and data that failed to meet a normal distribution were expressed by the median (quartile) [*M* (*q*25–*q*75)]. A Wilcoxon sample rank sum test was also used. For the sex comparison, a Pearson test was performed with enumeration data being expressed as number of cases (percentage) and chi-squared test used for comparisons between groups. The correlation analysis between FeNO and eosinophils percentage was performed by Spearson linear correlation. *P* values $<.05$ was considered statistically significant.

3. Results

A total of 110 patients with stable COPD were enrolled. As shown in Fig. 1, because of heart failure, bronchiectasis and uppering respiratory tract infection in the recent 2 weeks, 4 patients were excluded. Then patients were assigned into 2 groups: 53 cases of ACOS group and 53 cases of Non-ACOS group.

3.1. Demographics and clinical characteristics

There were no significant difference in sex, age, baseline medication of inhaled corticosteroid (ICS)+long-acting beta$_2$-agonist between the ACOS group and the Non-ACOS group ($P > .05$, respectively). Compared with Non-ACOS, the ACOS group had longer duration of disease, higher FEV_1 and FEV_1%, and lower smoking index, more frequency of long-acting ticholinergic (LAMA) use ($P < .01$, respectively), as shown in Table 1.

Comparison of FeNO levels between the 2 groups. The levels of FeNO were (37[24.5–53.0] ppb) in 53 cases of the ACOS group and (20[15.5–24.5] ppb) in the 53 cases of Non-ACOS group. Patients in the ACOS group had significantly higher FeNO levels

Table 1

Demographics and clinical characteristics in patients with ACOS and Non-ACOS (median [interquartile range]).

Groups	Number of cases (n)	Sex		Age, y	Duration of disease, y	FEV_1(L)	FEV_1%	Smoking index (package·year)
		Male	Female					
ACOS	53	44	9	68 (61.0–77.0)	21 (18.0–23.0)	1.10 (0.85–1.52)	56.00 (48.30–66.92)	19.00 (0.00–40.00)
Non-ACOS	53	47	6	71 (65.0–75.0)	14 (13.0–16.0)	0.91 (0.60–1.20)	43.00 (34.80–57.05)	40.00 (30.00–60.00)
$\chi^2/t/Z$ value		0.70		0.33	−7.61	−2.62	−4.52	1.34
P value		.40		.74	.00	.00	.00	.00

ACOS = asthma-COPD overlap syndrome, ICS = inhaled corticosteroid, LABA = long-acting beta$_2$-agonist, LAMA = long-acting ticholinergic.

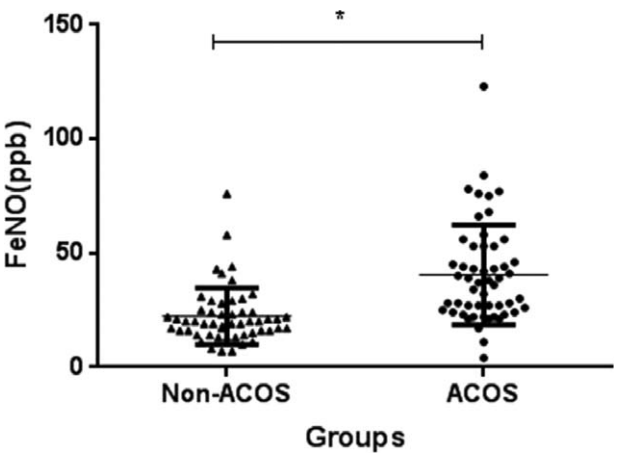

Figure 2. Comparison of FeNO levels in patients of the 2 groups. *: Comparison between ACOS group and Non-ACOS group ($P<.05$). ACOS = asthma-COPD overlap syndrome, FeNO = fractional exhaled nitric oxide.

than those in Non-ACOS group ($Z=-5.59$, $P=.00$), as shown in Fig. 2.

3.2. The ROC analysis of FeNO to ACOS phenotype diagnosis

Two groups' FeNO results by receiver operating curve (ROC curve) analysis and the Youden index was 0.51. The cut off value was 25.50 ppb, the sensitivity was 0.74, specificity was 0.77, the area under the ROC curve (AUC) was 0.815 ($P=.00$), indicating that FeNO has diagnostic value for ACOS phenotype, as shown in Fig. 3.

3.3. Multivariate logistic regression analysis

Multivariate logistic regression analysis was used to identify the independent factors between the ACOS group and the Non-ACOS group, duration of disease, FeNO, and smoking index all were independent factors of ACOS group ($P<.05$) as shown in Table 2.

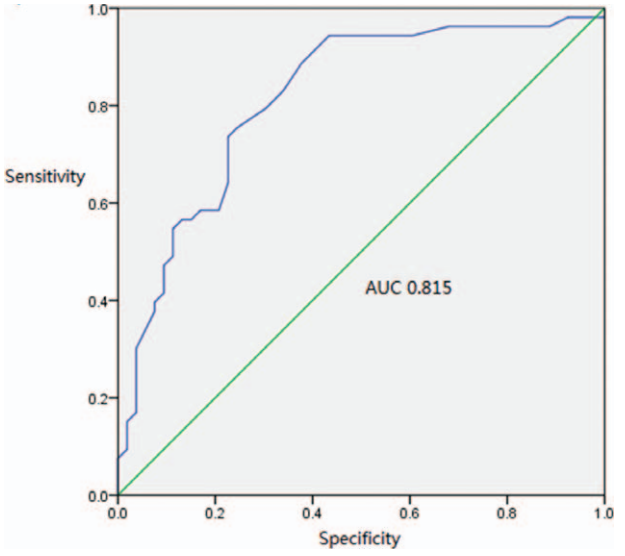

Figure 3. The ROC curve of FeNO to the ACOS phenotype diagnosis. Note: The area under the ROC curve (AUC): 0.815, $P=.00$. The Youden index was 0.51, FeNO = 25.50 ppb to achieve the best value, ROC curve = receiver operating curve.

Table 2

Multivariate logistic regression analysis in various factors.

Factors	β	Wald	*P*-value	OR (95% CI)
Smoking index (package ·year)	−0.087	5.66	.017	0.917 (0.853–0.985)
Duration of disease	0.733	17.439	<.001	2.083 (1.475–2.941)
FEV_1	1.406	1.362	.243	4.082 (0.385–43.478)
LAMA	1.122	1.320	.251	3.067 (0.453–20.833)
FeNO	0.058	5.423	.020	1.059 (1.009–1.112)

CI = confidence interval, LAMA = long-acting ticholinergic, FeNO = nitric oxide, OR = odds ratio.

3.4. Differential cell counts in induced sputum

Of the 61 patients who completed the induced sputum examination, 34 patients in the ACOS group and 27 patients in the Non-ACOS group. The percentage of eosinophils in patients with ACOS were over five times higher than those of patients with Non-ACOS ($P<.01$). The total white blood cell (WBC) counts showed no significant difference between the 2 groups. Neither did the percentages of neutrophils, macrophages, and lymphocytes between the 2 groups ($P>.05$), as shown in Table 3.

3.5. Correlation between differential cells counts in induced sputum and FeNO levels

FeNO levels in the 2 groups correlated positively with the percentage of eosinophils, $r=0.521$, $P=.00$. No significant correlations were found between FeNO levels and the total cell counts of WBC, percentages of neutrophils, macrophages, and lymphocytes (all $P>.05$), as show in Fig. 4.

4. Discussions

Asthma and COPD are commonly seen chronic respiratory diseases in the clinic. The 1961 "Dutch hypothesis" has suggested that asthma and COPD were different manifestations of the same disease caused by different environmental factors.[19] The common characteristics of these 2 diseases are airway inflammation and airflow limitation, but they have different airway inflammation characteristics.[11] Asthma is a chronic inflammatory reaction mediated by CD^{4+} T cells acting mainly on eosinophils. Meanwhile, COPD is a chronic inflammatory response mediated by CD^{8+}T cells. It is generally believed that ACOS patients are induced COPD on the basis of asthma, or on the basis of incomplete reversible airway obstruction, accompanied by a progressive increase in reversible obstructive symptoms or signs.[20] Therefore, ACOS should have the airway inflammatory characteristics of these 2 kinds of airway diseases, and it has important clinical significance for monitoring the level of airway inflammation in the diagnosis, treatment, prognosis, and recurrence of disease.

The 2012 Spanish ACOS Diagnostic Consensus also proposed that elevated sputum eosinophils could be used as main criteria for ACOS diagnosis. Our results showed that the level of eosinophils in the induced sputum in patients with the ACOS was significantly higher than those of patients with Non-ACOS. The total WBC count and percentages of neutrophils, macrophages,

Table 3

Differential cell counts in induced sputum in the 2 groups (median [interquartile range]).

Groups	Number of cases (n)	WBC ($\times 10^9$/L)	Macrophages (%)	Neutrophils (%)	Eosinophils (%)
ACOS	34	3.73 (1.31–13.01)	10.50 (7.50–22.00)	75.51 (57.37–87.87)	5.70 (1.50–17.62)
Non-ACOS	27	3.22 (2.22–8.04)	10.75 (2.75–18.00)	85.00 (72.00–89.00)	0.50 (0.00–1.00)
Z value		−0.89	0.43	1.70	−3.92
P value		.37	.66	.09	.00

ACOS = asthma-COPD overlap syndrome, WBC = white blood cell.

lymphocytes showed no statistically significant difference between the 2 groups, which is consistent with the results published by Kitaguchi et al.[21] They found that the sputum eosinophil level in the ACOS group was significantly higher than those in the simple COPD group. Airway inflammation is characterized by eosinophil infiltration in asthma, suggesting that the increased percentage of eosinophils in patients with ACOS indicates eosinophilic airway inflammatory response in ACOS is similar to asthma.

The induced sputum method is noninvasive and not limited to disease, but the operating procedures are quite stringent and complicated, since the results could be susceptible to

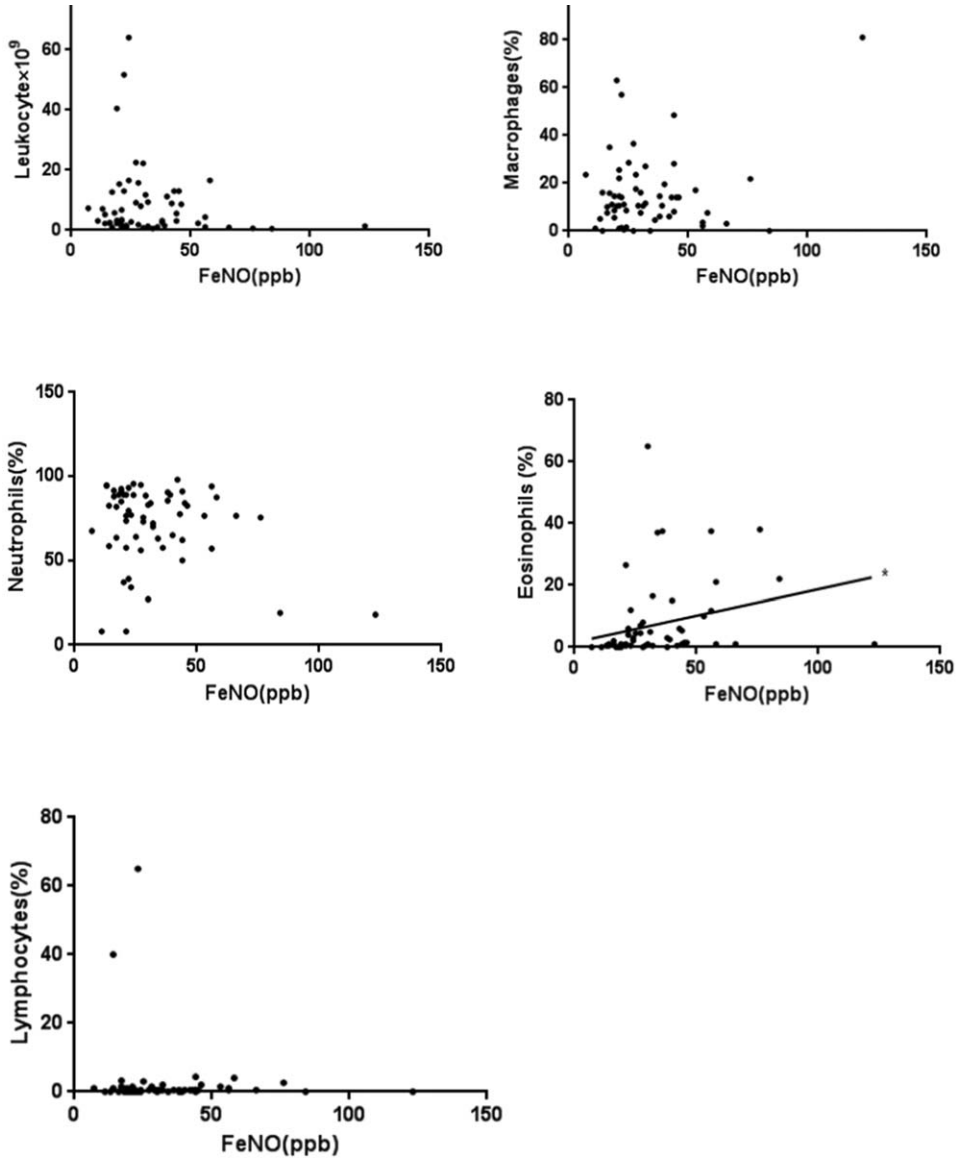

Figure 4. Scatter plots of correlation between the percentage of differential cells counts in induced sputum and FeNO in 2 groups. FeNO (27 [20.0–42.5] ppb) was positively correlated to the percentage of eosinophils (1.0 [0.5–6.5] %) in the 2 groups, $r = 0.521$ $P = .00$. No significant correlations were found between FeNO levels and the total cell counts of WBC, percentages of neutrophils, macrophages, and lymphocytes (*P* value were .43, .88, .38, .18, respectively). FeNO = fractional exhaled nitric oxide, WBC = white blood cell.

interference.[22] In the recent years, FeNO, as a noninvasive marker of airway inflammation, has been attracted wide attention. FeNO is more convenient, rapid, reproducible, and less painful than induced sputum analysis. The NO detected in exhaled air is produced by nitric oxide synthase catalyzing L-arginine in the airway epithelium. A variety of airway inflammations can lead to elevated NO; however, they are mostly dominated by eosinophilic inflammations.[23] In this study, after using multivariate logsitic regression, there was still difference in FeNO between the 2 groups after correcting duration of disease, FEV_1, LAMA, and smoking index: the levels of FeNO in patients with ACOS were significantly higher than those in patients with Non-ACOS, with a FeNO median in the ACOS group twice as high as that of the Non-ACOS group; FeNO was positively correlated with eosinophils in induced sputum. This result is consistent with the results of Seiichi[24]; Mi-jung[25] made a ROC analysis on the value of FeNO in the diagnosis of non-asthmatic eosinophilic bronchitis (NAEB), it was found that select the patients that cut point for >31.7 ppb had diagnostic value for NAEB. These results indicated that FeNO could reflect the levels of eosinophilic airway inflammation. Therefore, FeNO can be used as an indicator for evaluating airway inflammation in ACOS. Our results using ROC curve analysis showed that FeNO could be used for the diagnostic value of ACOS, the optimal cut off point was 25.50 ppb, and it had higher sensitivity and specificity. Chen et al[26] found that when the cutoff point was 21.5 ppb, the sensitivity was 70% and the specificity was up to 75%, which is similar to the results of this study.

Our study also showed that the duration of disease was longer in patients with ACOS compared with that of patients with Non-ACOS, possibly depending on the younger age onset of asthma. Generally, the age of asthma onset is 35 to 64 years old. Porsbjerg et al[27] have found that patients with asthma onset at over 64 years had faster FEV_1 decline with age and were more likely to progress into COPD. The FEV_1 and FEV_1% were higher in ACOS patients than those of patients with Non-ACOS, suggesting that the degree of pulmonary function impairment in patients with ACOS was milder, possibly due to the reversibility of airflow limitation. ACOS phenotype patients had better reaction to the ICS, and the lung function was significantly improved after inhaled ICS.[28] The airway stenosis in simple COPD may result from destruction of elastic fibers, decreased pulmonary elastic retraction force, and deformation of bronchioles and alveolar, followed by emphysema and alveolar wall damage.[29] The Non-ACOS group smoking index was significantly higher than that of ACOS group, it had been recognized that tobacco smoke was one of the causes of lung function damage.[30] In this study, although the numbers of patients using LAMA in Non-ACOS group were more than those of ACOS group, LAMA could only slow the decline in lung function, could not reverse or maintain lung function level, which had little effects on lung function to the 2 groups.[31,32] The results of this study were consistent with the results of Yoshiaki Kitaguch[33]: there were still significant differences in lung function between the 2 groups in the cases of no obvious difference in age and sex and elimination of drug interference.

In summary, the results indicated that FeNO over 25.50 ppb may be used as an auxiliary index for the diagnosis of ACOS phenotype, which may help clinicians to choose individualized treatment plan. Phenotypic research is still immature, the diagnostic criteria of ACOS still needs larger sample size and further more study to be established.

It cannot be denied that this study has limitations: all patients came from Respiratory Medicine Outpatient of Zhangzhou Municipal TCM Hospital, affiliated to Fujian University of Traditional Chinese Medicine. Case source is single, the representative aspect may have certain bias, and the sample size is small, only 106 cases. In order to make future research more accurately and comprehensively, larger sample of multi-center studies is necessary.

Author contributions

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Formal analysis: Yuanyuan Guo, Yanhong Liu, Huinuan Chen, Xiaohua Huang, Minli Hong.
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Methodology: Yuanyuan Guo, Chunlin Hong, Yanhong Liu, Huinuan Chen, Xiaohua Huang.
Resources: Yuanyuan Guo, Huinuan Chen.
Software: Yuanyuan Guo.
Supervision: Minli Hong.
Validation: Yuanyuan Guo, Chunlin Hong, Yanhong Liu, Huinuan Chen, Minli Hong.
Visualization: Yuanyuan Guo, Minli Hong.
Writing – original draft: Yuanyuan Guo, Minli Hong.
Writing – review and editing: Yuanyuan Guo, Minli Hong.

References

[1] Wedzicha JA. The heterogeneity of chronic obstructive pulmonary disease. Thorax 2000;55:631–2.
[2] Standards for the diagnosis, care of patients with chronic obstructive pulmonary diseaseAmerican Thoracic Society. Am J Respir Crit Care Med 1995;152:77–121.
[3] Han MK, Agusti A, Calverley PM, et al. Chronic obstructive pulmonary disease phenotypes: the future of COPD. Am J Respir Crit Care Med 2010;182:598–604.
[4] Soler-Cataluña JJ, Cosío B, Izquierdo JL, et al. Consensus document on the overlap phenotype COPD-asthma in COPD. Arch Bronconeumol 2012;48:331–7.
[5] Global strategy for asthma management and prevention; 2014 (revision). 2017. Available at: www.ginasthma.org.
[6] Barrencheguren M, Esquinas C, Miravitlles M. The asthma-chronic obstructive pulmonary disease overlap syndrome (ACOS): oppprtunities and challenges. Curr Opin Pulm Med 2015;21:74–9.
[7] Zeki AA, Schivo M, Chan A, et al. The asthma-COPD overlap syndrome: a common clinical problem in the elderly. J Allergy (Cairo) 2011;2011: 861926.
[8] Soriano JB, Davis KJ, Coleman B, et al. The proportional Venn diagram of obstructive lung disease: two approximations from the United States and the United Kingdom. Chest 2003;124:474–81.
[9] Bourdin A, Serre I, Flamme H, et al. Can endobronchial biopsy analysis be recommended to discriminate between asthma and COPD in mutine practice? Thorax 2004;59:488–93.
[10] Contoli M, Baraldo S, Marku B, et al. Fixed aiflow obstruction due to asthma or chronic obstructive pulmonary disease: 5-year follow-up. J Allergy Clin Immunol 2010;125:830–7.
[11] Fabbri LM, Micaela R, Lorenzo C, et al. Differences in airwayinflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2004;167:418–24.
[12] Chunlin H, Huinuan C, Min-li H, et al. Induced sputum cytology analysis in different cause of chronic cough. Chin J Postgrad Med 2010;33:32–4.
[13] Petsky HL, Kew KM, Turner C, et al. Exhaled nitric oxide levels to guide treatment for adults with asthma. Cochrane Database Syst Rev 2016; CD011440.

[14] James A, Hedlin G. Biomarkers for the phenotyping and monitoring of asthma in children. Curr Treat Options Allergy 2016;3:439–52.
[15] Karampitsakos T, Gourgoulianis KI. Asthma-COPD Overlap Syndrome (ACOS): Single disease entity or not? Could exhaled nitric oxide be a useful biomarker for the differentiation of ACOS, asthma and COPD? Med Hypotheses 2016;6:20–3.
[16] Guidelines on diagnosis and treatment of chronic obstructive pulmonary disease (2013 Revision). Chin J Tuberculosis Respir Dis 2013;36:11-0.
[17] American Thoracic, Society, European Respiratory SocietyATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005;171:912–30.
[18] Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease (updated 2015) [EB/OL]. [2015-01]. 2017. Available at: http://www.goldcopd.org/guidelines-globalstrategy-for-diagnosis-management.html.
[19] Nakawah MO, Hawkins C, Barbandi F. Asthma, chronic obstructive pulmonary disease (COPD), and the overlap syndrome. J Am Board Fam Med 2013;26:470–7.
[20] Hardin M, Cho M, McDonald ML, et al. The clinical and genetic features of COPD-asthma overlap syndrome. Eur Respir J 2014;44:341–50.
[21] Kitaguchi Y, Komatsu Y, Fujimoto K, et al. Sputum eosinophilia can predict responsiveness to inhaled corticosteroid treatment in patients with overlap syndrome of COPD and asthma. Int J Chron Obstruct Pulmon Dis 2012;7:283–9.
[22] Bartoli ML, Bacci E, Cianchetti S, et al. Some factors influencing quality of spontaneous or induced sputum for inflammatory cell analysis. Monaldi Arch Chest Dis 2007;67:81–3.
[23] Guo FH, Comhair SA, Zheng S, et al. Molecular mechanisms of increased nitric oxide (NO) in asthma: evidence for transcriptional and post-translational regulation of NO synthesis. J Immunol 2000;164: 5970–80.
[24] Kobayashi S, Hanagama M, Yamanda S, et al. Inflammatory biomarkers in asthma-COPD overlap syndrome. Int J Chron Obstruct Pulmon Dis 2016;11:2117–23.
[25] Oh MJ, Lee JY, Lee BJ, et al. Exhaled nitric oxide measurement is useful for the exclusion of nonasthmatic eosinophilic bronchitis in patients with chronic cough. Chest 2008;134:990–5.
[26] 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.
[27] Porsbjerg C, Lange P, Ulrik CS. Lung function impairment increases with age of diagnosis in adult onset asthma. Respir Med 2015;109: 821–7.
[28] Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. Am J Respir Crit Care Med 2001;164(10 pt 2):S28–38.
[29] Yoshiaki K, Yoshimichi K, Keisaku F, et al. Sputum eosinophilia can predict responsiveness to inhaled corticosteroid treatment in patients with overlap syndrome of COPD and asthma. Int J Chronic Obstruct Pulmonary Dis 2012;7:283.
[30] Warnier MJ, van Riet EE, Rutten FH, et al. Smoking cessation strategies in patients with COPD. Eur Respir J 2013;41:727–34.
[31] Tashkin D, Kesten S. Long-term treatment benefits with tiotropium in COPD patients with and without short-term bronchodilator responses. Chest 2003;123:1441–9.
[32] Decramer M, Celli B, Kesten S, et al. Effect of tiotropium on outcomes in patients with moderate chronic obstructive pulmonary disease (UPLIFT): a prespecified subgroup analysis of a randomised controlled trial. Lancet 2009;374:1171–8.
[33] Yoshiaki K, Yoshimichi K, Keisaku F, et al. Sputum eosinophilia can predict responsiveness to inhaled corticosteroid treatment in patients with overlap syndrome of COPD and asthma[J]. Int J Chron Obstruct Pulmon Dis 2012;7:283–9.