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ORIGINAL RESEARCH

Clinical Utility of Central and Peripheral Airway Nitric Oxide in Aging Patients with Stable and Acute Exacerbated Chronic Obstructive Pulmonary Disease

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Purpose: Exhaled nitric oxide has been used as a marker of airway inflammation. The NO concentration in the central and peripheral airway/alveolar can be measured by a slow and fast exhalation flow rate to evaluate inflammation in different divisions within the respiratory tract. We hypothesized that $FeNO_{200}$ (exhaled NO at a flow rate of 200mL/s) could be used as an evaluation tool for peripheral airway/alveolar inflammation and corticosteroid therapy in chronic obstructive pulmonary disease (COPD) patients.

Methods: We recruited 171 subjects into the study: 73 healthy controls, 59 stable COPD patients, and 39 acute exacerbations of COPD (AECOPD) patients. Exhaled nitric oxide ($FeNO_{50}$ (exhaled NO at a flow rate of 50mL/s)), $FeNO_{200}$ and CaNO (peripheral concentration of NO/alveolar NO) and clinical variables including pulmonary function, COPD Assessment Test (CAT), C-reactive protein concentration (CRP) and circulating eosinophil count were measured among the recruited participants. $FeNO_{50,}$ $FeNO_{200}$ and CaNO were repeatedly evaluated in 39 AECOPD patients after corticosteroid treatment.

Results: $FeNO_{200}$ was significantly higher in stable COPD and AECOPD patients than in healthy controls. Nevertheless, CaNO could not differentiate COPD from healthy controls. No correlation was found between circulating eosinophil counts or FEV1 and exhaled nitric oxide ($FeNO_{50,}$ $FeNO_{200,}$ CaNO) in COPD patients. For AECOPD patients, 64% of patients had eosinophil counts >100 cells/μL; 59% of patients had $FeNO_{200}$ >10 ppb; only 31% of patients had $FeNO_{50}$ > 25 ppb. Among AECOPD patients, the high $FeNO_{50}$ and $FeNO_{200}$ groups' levels were significantly lower than their baseline levels, and significant improvements in CAT were seen in the two groups after corticosteroid treatment. These implied a good corticosteroid response in AECOPD patients with $FeNO_{200}$>10ppb.

Conclusion: $FeNO_{200}$ is a straightforward and feasible method to evaluate the peripheral NO concentration in COPD. FeNO200 can be a type 2 inflammation biomarker and a useful tool for predicting corticosteroid therapy in COPD.

Keywords: exhaled nitric oxide, chronic obstruction pulmonary disease, corticosteroid, biomarker

Introduction

Chronic obstructive pulmonary disease (COPD) is a complex polyclinic lung disease characterized by an abnormal inflammatory and a progressive condition with declining lung function.[1] Although airway inflammation in COPD is generally considered to be caused by type 1 immune response, type 2 airway inflammation

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can also occur in some COPD patients during stable or exacerbation state.[2] Some studies have found that some COPD patients have gene expression of type 2 inflammation in the airway, and these patients have a good response to corticosteroid.[3] In recent years, there have been a large number of studies on the role of type 2 biomarkers in COPD, including eosinophils, IgE and $FeNO_{50}$.[4] Nitric oxide (NO) is biosynthesized from L-arginine and oxygen by the enzyme NO synthase (NOS) endogenously, fractional concentration of exhaled nitric oxide at a flow rate of 50mL/s ($FeNO_{50}$) is a known marker of airway inflammation. As a noninvasive, convenient and highly reproducible method for assessing airway inflammation, FeNO measurement has been used to evaluate type 2 inflammation of asthma and guide anti-inflammatory treatment.[5,6] However, the role of $FeNO_{50}$ in COPD remains controversial.[7–10]

According to the latest technical standard published by the European Respiratory Society (ERS) in 2017, $FeNO_{50}$ (usually abbreviated as FeNO, representing the exhaled NO value at the flow rate of 50mL/s) mainly reflects large airway inflammation from bronchi to respiratory bronchioles, but cannot reflect small airway inflammation.[11] COPD is a respiratory disease characterized by chronic inflammation of the small airway. As a marker of peripheral/small airway inflammation, more and more studies began to pay attention to the clinical value of CaNO (concentration of alveolar NO) in COPD patients. Several studies reported that CaNO was increased in COPD patients and was correlated with a single nitrogen washout curve (dN2) and diffusion capacity for carbon monoxide (DLCO).[12–17] Nonetheless, some researchers found that there was no difference in CaNO between COPD patients and healthy population.[18] In addition, whether CaNO could guide the treatment of corticosteroid in COPD patients needs further study.

The estimation of CaNO requires three exhalations at three different flow rates that follow the conventional linear regression model. The procedure is complex, especially for AECOPD patients, it is challenging to complete.[16] Therefore, it is necessary to have a simpler and more convenient index than CaNO, but it can also reflect peripheral/small airway inflammation. In 2014, Peter J Barnes found that $FeNO_{200}$ (fractional concentration of exhaled nitric oxide at a flow rate of 200 mL/s) is proportional to CaNO. By measuring the exhaled NO at the flow rates of 50 mL/s and 200 mL/s, the inflammation of central and peripheral airways can be distinguished.[19] Therefore, $FeNO_{200}$ (fractional concentration of exhaled NO at a flow rate of 200 mL/s) was used as it maximizes the alveolar fraction of eNO in hereditary hemorrhagic telangiectasia, hepatopulmonary syndrome, and liver cirrhosis.[20–22] However, the role of $FeNO_{200}$ in patients with COPD has not been studied.

Our prospective study measured pulmonary function parameters and exhaled nitric oxide ($FeNO_{50}$, $FeNO_{200}$, and CaNO) in COPD patients with different stages and controls. For AECOPD patients, we compared these parameters before and after corticosteroid treatment. The purpose of this study was to evaluate a change in peripheral small airway/alveolar sites of NO detected by $FeNO_{200}$ and CaNO in COPD. Besides, whether $FeNO_{200}$ or CaNO could be used as an evaluation tool for corticosteroids therapy in AECOPD patients was further explored.

Materials and Methods

Study Subjects

Stable COPD patients and AECOPD patients were diagnosed based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2017 guidelines. Healthy subjects (HS) with normal lung function as controls were included in the study. All participants were restricted to adults 60 years and older. The physician prescribed the therapy of AECOPD. Patients enrolled at the Department of Respiratory Medicine at the Wuxi People's Hospital from November 2017 to December 2019.

For all participants with a history of asthma or other respiratory diseases were excluded from the study. The main exclusion criteria were treated with systemic corticosteroids or antibiotics 4 weeks before admission. The hospital Ethics Committee approved the study. We fully abide by the guidelines in the Helsinki declaration and written informed consent was obtained from all participants enrolled in the study.

Study Design

Lung function, exhaled nitric oxide, the white blood cell count (WBC), and blood C-reactive protein (CRP) concentration were measured among the controls and COPD patients. For AECOPD patients, methylprednisolone 40mg/day, intravenous administration for 1 week was given. Moreover, before discharged from the hospital, lung function and exhaled nitric oxide would be measured again in AECOPD patients.

Exhaled NO Measurement

Exhaled NO was measured using the Nano Coulomb Breath Analyzer (Sunvou-CA2122, Wuxi, China), in line with the American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations.[23] One hour before the test, eating, smoking, drinking, strenuous exercise or pulmonary function test were prohibited.

Ten healthy controls, ten stable COPD patients, and ten AECOPD patients participated in the preliminary clinical trial. They were required to exhale via a mouthpiece at multiple flow rates: 100, 200, 350mL/s. At each flow rate, the mean value was calculated. FeNO50 was measured at the single flow rate of 50mL/s. CaNO was estimated with a mathematical approach based on a two-compartment linear model published by Tsoukias et al.[24] However, the success rate of measurement at a flow rate of 350 mL/s was only 60% for AECOPD patients (Table 1).

A simplified estimation method of alveolar nitric oxide was developed to improve the success rate of measurement. Subjects were informed about inhaling NO-free air and exhaling via a mouthpiece at two constant flow rates: 50, 200mL/s. $FeNO_{50}$ and $FeNO_{200}$ were recorded. CaNO was calculated based on the linear model published by ERS:

FeNO = CaNO + JawNO/VE

FeNO (fractional concentration of exhaled NO) is recorded in ppb (1ppb = 1x10-9mol/L). The exhalation flow rate is given as a subscript in mL/s. A flow rate of 50 mL/s is written $FeNO_{50}$ and a low rate of 200 mL/s is written $FeNO_{200}$. FeNO is a flow rate dependent index. The smaller the flow rate is, the higher the value is, the better it can reflect NO in a large airway; the higher the flow rate, the smaller the value, the better it can reflect NO in a small airway. JawNO, the total flux of NO in the conducting airway compartment (nl/s), is not affected by the flow rate and only reflects the inflammation of the central/large airway. CaNO, the concentration of alveolar NO, is not affected by the flow rate and only reflects the inflammation of peripheral/small airway.

Table 1 The Success Rate of Exhaled Nitric Oxide Measurement at Different Expiratory Flow Rates

Exhalation Flow Rates, mL/s	50	100	200	350
Control	100%	100%	100%	90%
Stable COPD	100%	100%	90%	70%
AECOPD	100%	100%	90%	60%

Statistical Analysis

SPSS software vsrsion22.0 was used to process data. All continuous variables were checked for normal distribution by Kolmogorov–Smirnov normality test. Normally distributed variables are expressed as mean ± standard deviation, and skewed variables are expressed as the median (interquartile range, IQR). The significance of the difference between the two groups was assessed with a two-tailed Manne–Whitney test (*t*-test). The differences between multiple groups were evaluated with the nonparametric Kruskal–Wallis test (ANOVA) with Dunn post-test. The chi-square test was used to compare categorical variables between the two groups. Statistical significance was considered to exist when $P<0.05$.

Results

Subject Characteristics

A total of 98 COPD patients (59 stable COPD patients, 39 AECOPD patients) and 73 healthy controls were enrolled in this study. The characteristics of the study patients are shown in Table 2. There were no statistical differences in terms of age, blood eosinophil percentage, and eosinophil counts. Elevated CRP concentration was seen in COPD patients. Pulmonary function parameters were significantly higher in healthy controls than in COPD patients. In the stable COPD and AECOPD patients, lung function parameters and maintenance therapy were similar. There was no significant difference in the distribution of disease severity between the COPD and AECOPD patients.

Increased Exhaled Nitric Oxide in Different Stages of COPD

$FeNO_{200}$ elevated in patients with stable COPD [median: 11.0 ppb, IQR: (9.0, 15.0) ppb; $P<0.05$] and AECOPD patients [meidan: 11.0 ppb, IQR: (10.0, 14.0) ppb; $P<0.05$] compared with healthy controls [median: 9.0 ppb, IQR: (6.0, 11.0) ppb]. $FeNO_{50}$ increased in patients with stable COPD [median: 22.0 ppb, IQR: (17.0, 30.0) ppb; $P<0.05$] and exacerbated COPD patients [median: 21.0 ppb, IQR: (18.0, 28.0) ppb; $P<0.05$] compared with healthy controls [median: 20.0 ppb, IQR: (15.0, 25.0) ppb]. However, there was no significant difference in $FeNO_{50}$ and $FeNO_{200}$ between stable and exacerbated COPD patients. CaNO demonstrated no significant difference among control subjects [median: 4.4 ppb, IQR: (1.5, 6.7) ppb], stable [median: 5.9 ppb, IQR: (3.5, 8.2) ppb] and exacerbated COPD patients [median: 5.4 ppb, IQR: (2.6, 8.5) ppb] (Figure 1).

Table 2 The Clinical Characteristics of the Patients and Control

	Control	Stable COPD	AECOPD	P-value	Paired Comparison Results
Demographics					
Numbers	73	59	39		
Age (years)	67±6	69±7	68±7	0.055	—-
Gender (% male) *	54(74%)	57(97%)	37(95%)	0.000	Control, Stable COPD>AECOPD
Current smoker*	23(32%)	34(56%)	18(45%)	0.018	Stable COPD>AECOPD>Control
Comorbiditis					
Hypertension	4	17	15	—-	
Diabetes	3	4	2	—-	
Blood test					
Eosinophil count/ul	0.10 (0.07, 0.17)	0.14 (0.05,0.27)	0.15 (0.04,0.21)	0.571	—-
Eosinophil count≥100cells/ul,%	-	47%	64%		
CRP, mg/l	0.8 (0.5,2.1)	2.8 (0.5,9.0)	4.0 (1.0,10.9)	0.002	AECOOD>Stable COPD>Control
Pulmonary function					
VC MAX	2.98±0.71	2.68±0.67	2.51±0.78	0.000	Control>Stable COPD, AECOPD
FEV1/FVC	80.74 (72.40,94.17)	50.11±9.43	48.9±11.5	0.000	Control>Stable COPD, AECOPD
FEV1% pred	91.79±18.80	46.81±16.00	39.6 (30.1,54.1)	0.000	Control>Stable COPD, AECOPD
PEF	6.48±1.98	3.3 (1.62,7.92)	2.9 (2.0,3.9)	0.000	Control>Stable COPD, AECOPD
FEF25	6.27±2.04	1.21 (0.55,3.82)	0.83 (0.58,1.78)	0.000	Control>Stable COPD, AECOPD
FEF50	5.03±2.13	0.61 (0.26,1.36)	0.42 (0.28,0.76)	0.000	Control>Stable COPD, AECOPD
FEF75	2.64±1.48	0.20 (0.10,0.44)	0.19 (0.15,0.29)	0.000	Control>Stable COPD, AECOPD
Treatment					
ICS, N	NA	43	39	0.241	
ICS dose,ug BUD	NA	640(640–800)	640(640–800)	0.930	
LABA, N	NA	46	37	1.000	
LAMA, N	NA	51	32	0.420	
Oral theophylline, N	NA	12	13	0.210	

Notes: Data are presented as mean±standard deviation, median (interquartile range), or number of subjects (proportion); *Mantel-Haenszel chi-square test for Gender and Current smokers; Kruskal–Wallis test for all other variables; Comparisons made between Control, Stable COPD and AECOPD categories .

Abbreviations: CRP, blood C-reactive protein; VT, tidal volume; VC MAX, VC, vital capacity; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity. PEF, peak expiratory flow; FEF25, forced expiratory flow when 25% of vital capacity is exhaled; ICS, inhaled corticosteroid; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist.

There was a positive correlation between $FeNO_{50}$ and CRP (r=0.384, p<0.05) in stable COPD patients. $FeNO_{200}$ was associated with CRP (r=0.335, p<0.05) as well. CaNO did not correlate with CRP (p>0.05). Forty-seven percent stable COPD patients and 64% AECOPD patients with blood eosinophil counts ≥100cells/ul. No correlation was found between exhaled nitric oxide ($FeNO_{50}$, $FeNO_{200}$, CaNO) and eosinophils (P>0.05). In AECOPD patients, there was no correlation between exhaled nitric oxide and eosinophils and CRP (p>0.05).

Effect of Corticosteroids on Exhaled Nitric Oxide in AECOPD Patients

Exhaled NO was repeatedly measured in 39 patients with AECOPD before discharge from the hospital following adequate corticosteroid treatment. During hospitalization, all patients were treated continuously with systemic corticosteroids. There were significant improvements in $FeNO_{50}$, lung function (FEV 1%, PEF, FEF25, FEF50, FEF75) (Table 3).

$FeNO_{200}$ >10ppb Predicted a Good Corticosteroid Response in AECOPD

We grouped all patients according to the cut-off point of $FeNO_{50}$, $FeNO_{200}$ and CaNO. According to the clinical guideline published by ATS in 2011, $FeNO_{50}$ values greater than 25ppb indicate eosinophilic inflammation and the likelihood of corticosteroid responsiveness. No guidelines have published cut-off points for $FeNO_{200}$ and CaNO. Based on the literature, the normal values of $FeNO_{200}$ and CaNO in healthy people do not exceed 7.4 ppb and 4.7 ppb, so we use 10ppb and 5ppb as the cut-off point.[20,25,26]

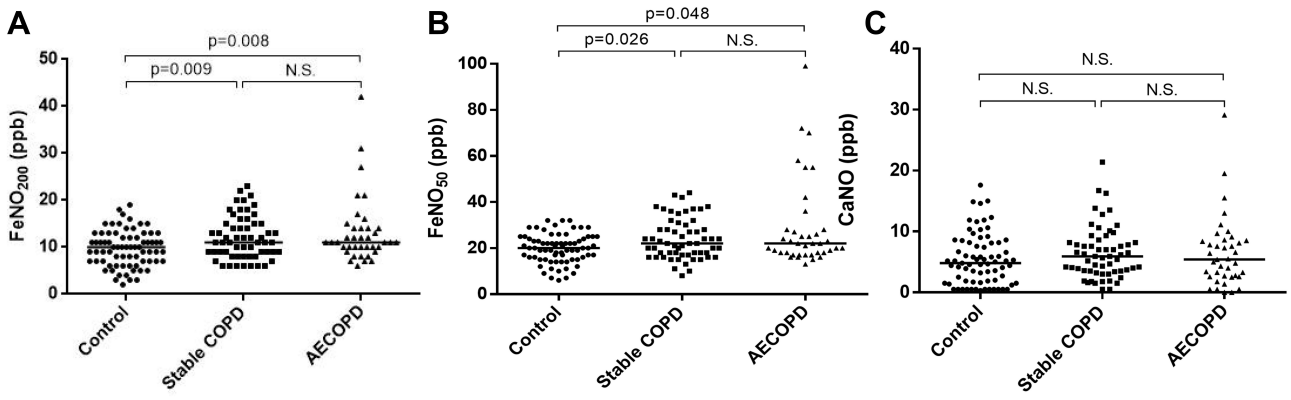

Figure 1 Exhaled nitric oxide in healthy controls, stable and exacerbated COPD patients. $FeNO_{50}$ increased in stable and exacerbated COPD patients (**A**). $FeNO_{200}$ increased in stable and exacerbated COPD patients (**B**). There was no significant difference of CaNO in healthy controls, stable and exacerbated COPD patients (**C**).

There were 11 patients with initial $FeNO_{50}$> 25 ppb, $FeNO_{50}$ decreased significantly from 51.7±22.7 ppb to 30.3±9.0 ppb ($p<0.05$) after a week of corticosteroid treatment (Figure 2A). For 28 patients with initial $FeNO_{50} \leq 25$ ppb, $FeNO_{50}$ did not change significantly (19.0±3.2 vs 20.0±9.3 ppb; p=0.764) (Figure 2B). There were 23 patients with initial $FeNO_{200}$> 10 ppb, $FeNO_{200}$ decreased from 16.1±7.8 to 12.2±5.2 after treatment ($p<0.05$) (Figure 2C). And for 16 patients with initial $FeNO_{200} \leq 10$ ppb, there was no significant change after treatment (8.7±1.4 vs 10.3±4.3ppb; p=0.112) (Figure 2D). There were 18 patients with initial CaNO >5 ppb and 21 patients ≤5 ppb, and no significant change was found in neither group (10.3±5.9 vs 7.4 ±5.0 ppb, p=0.181; 2.6±1.5 vs 4.5±5.7 ppb, p=0.148; Figure 2E and F).

The patients in the high $FeNO_{50}$ group ($FeNO_{50}$> 25 ppb) had greater improvement in $FeNO_{50}$ and CAT than the low $FeNO_{50}$ group ($FeNO_{50} \leq$ 25 ppb) (Table 4). Similar results were found between the high $FeNO_{200}$ group ($FeNO_{200}$ > 10 ppb) and low $FeNO_{200}$ group ($FeNO_{200} \leq$ 10 ppb) (Table 5). No improvement was observed in both high and low CaNO groups (Table 6).

No Relationship Between COPD Severity and Exhaled Nitric Oxide

According to the GOLD criteria, four patients were categorized as GOLD stage1 (FEV1% pred≥80%), 32 as GOLD stage 2 (80% > FEV1% pred ≥50%), 41 as GOLD stage 3 (50% > FEV1% pred ≥30%) and 21 as stage 4 (FEV1% pred <30%).

The increase of CaNO from GOLD1-2 to GOLD3, GOLD 3 to GOLD 4 was insignificant, but there was a significant increase from GOLD 1–2 to GOLD 4 (Figure 3C). And no difference was found between different GOLD stages in $FeNO_{50}$ and $FeNO_{200}$ (Figure 3A and B).

No correlations were found between $FeNO_{50}$, $FeNO_{200}$, CaNO and FEV1 ($p>0.05$).

Table 3 Changes in Exhaled NO and Pulmonary Function in AECOPD Patients After Treatment

	Before	After	Difference (After-Before)	*p* value
$FeNO_{50}$	22.0 (18.0,28.0)	22.7±9.5	−3.0 (−10.0,3.0)	0.037
$FeNO_{200}$	11.0 (10.0,14.0)	10.0 (8.0,14.0)	0.0 (−5.0,2.0)	0.118
CaNO	5.4 (2.7,8.5)	4.6 (2.5,6.8)	−0.2 (−2.6,2.3)	0.796
CAT	18.00(15.00,32.00)	19.00(11.00,26.00)	−3.00(−6.00,-1.00)	0.000
FEV1	0.90(0.74,1.46)	1.07(0.91,0.59)	0.12(−0.04,0.25)	0.000
FEV1/FVC	48.9±11.5	52.1±14.3	0.52 (−2.2,5.0)	0.048
FEV1%	39.6 (30.1,54.1)	49.7±18.2	4.3(−2.7,9.8)	0.003
PEF	2.9 (2.0,3.9)	3.7 (2.6,4.8)	0.35±1.43	0.013
FEF25	0.83 (0.58,1.78)	1.47 (0.72,2.60)	0.13 (−0.10–0.65)	0.000
FEF50	0.42 (0.28,0.76)	0.58 (0.37,1.11)	0.06 (−0.05–0.26)	0.006
FEF75	0.19 (0.15,0.29)	0.24 (0.16,0.34)	0.03±0.16	0.039

Notes: Data are presented as mean±standard deviation or median (interquartile range); Kruskal–Wallis test for all other variables.

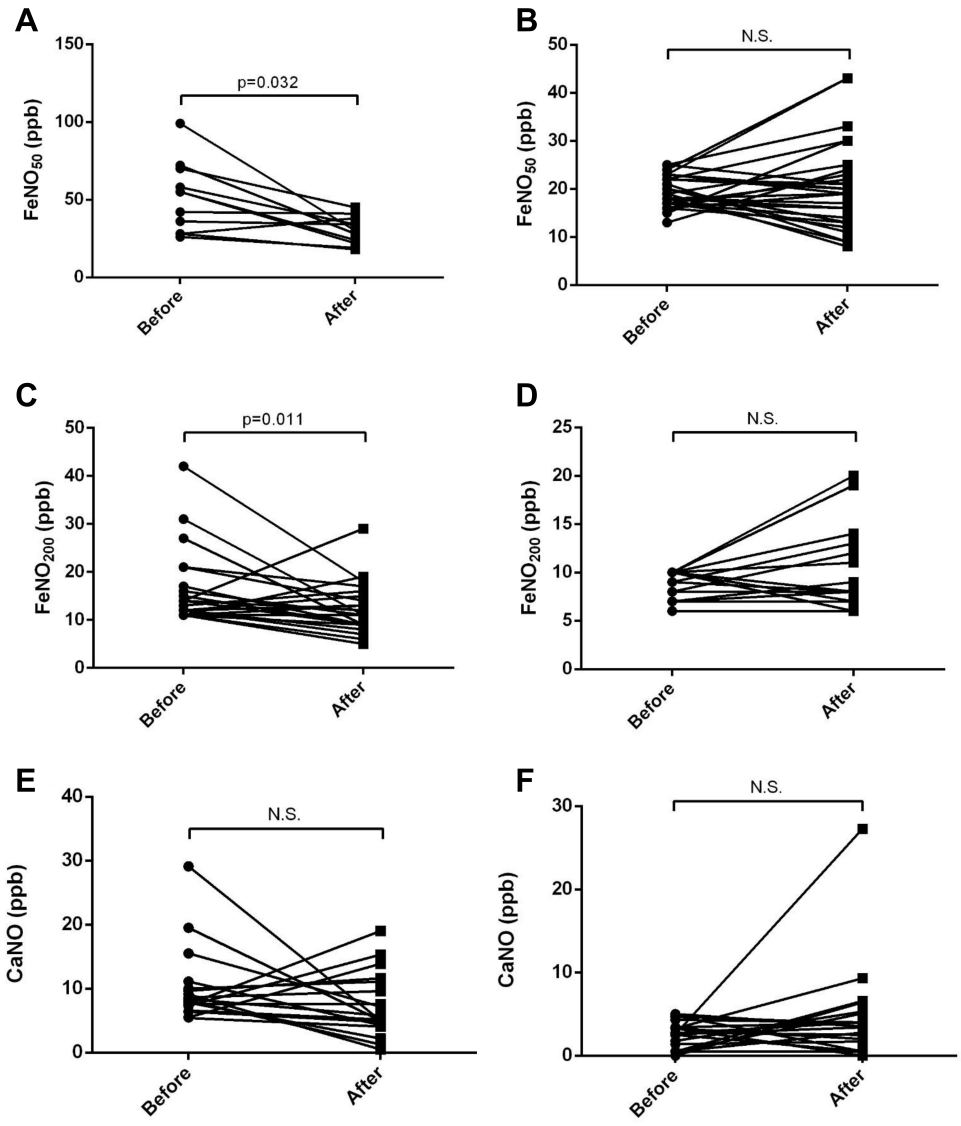

Figure 2 Changes in $FeNO_{50}$, $FeNO_{200}$ and CaNO in different groups. $FeNO_{50}$ in patients with initial $FeNO_{50}$> 25 ppb decreased after treatment (**A**), $FeNO_{50}$ in patients with initial $FeNO_{50}$ ≤ 25 ppb did not change after treatment (**B**), $FeNO_{200}$ in patients with initial $FeNO_{200}$> 10 ppb decreased after treatment (**C**), $FeNO_{200}$ in patients with initial $FeNO_{200}$ ≤ 10 ppb did not change after treatment (**D**), CaNO in patients with initial CaNO > 5 ppb did not change after treatment (**E**), CaNO in patients with initial CaNO ≤ 5 ppb did not change after treatment (**F**).

Discussion

Invasive sampling, such as lung biopsy or bronchoalveolar lavage (BAL), poses a risk to COPD patients, making it difficult to assess small airway and alveoli inflammation directly. Exhaled NO has been used as a noninvasive biomarker of airway inflammation since it was discovered. According to ERS guideline, $FENO_{50}$ = CANO+JawNO/50 at the flow rate of 50mL/s and $FENO_{200}$ = CANO +JawNO/200 at the flow rate of 200mL/s. JawNO is the NO flux in the large airway, which reflects the NO production and inflammation in the large airway, and is not affected by the flow rate; similarly, CANO is the alveolar NO concentration, which completely reflects the small airway inflammation and is not affected by the flow rate. Therefore, $FENO_{50}$ reflects more JawNO, that is, NO concentration in large airway, while $FeNO_{200}$ reflects more CANO, that is, NO concentration in small airway. We observed that $FeNO_{200}$ elevated in both stable and acute exacerbated COPD patients. Further subgroup analysis implied that $FeNO_{200}$ in AECOPD patients with greater than 10ppb decreased after systemic corticosteroid therapy. However, no similar changes in CaNO were found. As we know, this is the first report of assessing the role of $FeNO_{200}$ in COPD and may have important clinical significance.

Table 4 The Difference from Baseline in $FeNO_{50}$, CAT and Pulmonary Function Test

Variables	High $FeNO_{50}$	Low $FeNO_{50}$	P-value
$FeNO_{50}$	−24.00(−33.00,-2.00)	−1.50(−4.75,6.75)	0.018
CAT	−6.00(−9.00,-4.00)	−2.50(−4.00,0.50)	0.031
FEV1/FVC	6.48(−0.74,8.68)	2.44(−0.96,8.74)	0.286
FEV1% pred	2.70(−0.90,16.70)	6.35(−0.88,9.78)	0.340
FEV1	0.09(−0.05,0.28)	0.17(−0.05,0.26)	0.370
PEF	0.20(−0.42,1.40)	0.57(0.17,0.96)	0.676
PEF25	0.12(−0.15,0.72)	0.18(0.00,0.48)	0.906
PEF50	0.12(−0.06,0.43)	0.05(−0.02,0.23)	0.427
PEF75	0.06(0.01.0.18)	0.03(−0.01,009)	0.427

Notes: Data are presented as median (interquartile range); Kruskal–Wallis test for all other variables.

We discovered that $FeNO_{200}$ in COPD and AECOPD patients were higher than those in the healthy control group, reflecting the peripheral airway inflammation in COPD patients. Also, our research showed that $FeNO_{200}$ was correlated with CRP in stable COPD patients, which was consistent with some findings in CaNO.[15,16]

Table 5 The Difference from Baseline in $FeNO_{200}$, CAT and Pulmonary Function Test

Variables	High $FeNO_{200}$	Low $FeNO_{200}$	P-value
$FeNO_{200}$	−4.00(−6.00,1.00)	0.50(−0.75,4.00)	0.017
CAT	−4.00(−6.00,-3.00)	−1.00(−3.75,0.50)	0.031
FEV1/FVC	2.62(−1.99,7.83)	3.54(−0.17,13.76)	0.542
FEV1% pred	3.50(−2.30,11.40)	5.90(1.10,9.43)	0.803
FEV1	0.09(−0.04,0.24)	0.19(−0.05,0.31)	0.921
PEF	0.72(−0.05,1.09)	0.34(0.07,0.73)	0.176
PEF25	0.13(−0.10,0.50)	0.32(0.09,0.71)	0.611
PEF50	0.05(−0.03,0.25)	0.13(−0.02,0.28)	0.863
PEF75	0.02(−0.01,0.13)	0.04(0.00,0.10)	0.922

Notes: Data are presented as median (interquartile range); Kruskal–Wallis test for all other variables.

Table 6 The Difference from Baseline in CaNO, CAT and Pulmonary Function Test

Variables	High CaNO	Low CaNO	P-value
CaNO	−1.50(−7.18,1.30)	0.30(−1.30,4.05)	0.107
CAT	−3.50(−5.00,-5.00)	−3.00(−6.00,-1.00)	0.951
FEV1/FVC	0.67(−1.81,4.47)	6.48(0.22,14.42)	0.135
FEV1% pred	3.50(−1.35,9.25)	6.30(0.00,16.40)	0.407
FEV1	0.17(−0.07,0.27)	0.09(−0.04,0.25)	0.621
PEF	0.67(0.11,1.02)	0.34(−0.42,0.34)	0.873
PEF25	0.13(−0.13,0.40)	0.20(−0.15,0.20)	0.135
PEF50	0.04(−0.06,0.09)	0.25(−0.35,0.25)	0.010
PEF75	−0.01(−0.01,0.08)	0.04(−0.05,0.04)	0.232

Notes: Data are presented as median (interquartile range); Kruskal–Wallis test for all other variables.

Nevertheless, the same changes of CaNO were not found in this study. Our results are in line with a few studies, suggesting that CaNO did not differentiate healthy controls from COPD patients.[18,27] However, several studies have found that the CaNO of COPD and AECOPD patients were higher than those of healthy controls.[15,16] The inconsistency may be due to the use of different types of NO analyzers in these studies. CaNO varies with different flow rates, velocities, and various calculation models. Low, medium and high exhalation flow rates are needed for CaNO calculation, but this method is challenging to apply in COPD patients.[11] Our pre-test showed that nitric oxide measurement only had a 70% success rate at 300 mL/s expiratory flow rate. Thus, $FeNO_{200}$ can reflect peripheral airway inflammation more directly and accurately. Compared with the complex operation and calculation of CaNO, $FeNO_{200}$ is an effective and simple method to evaluate small airway inflammation, especially in AECOPD patients. A larger sample size is needed for further research.

The peripheral airways NO measured by $FeNO_{200}$ and the simplified CaNO were not elevated as we expected in AECOPD compared with the stable condition. Zsófia Lázár also reported the same results in CaNO.[16] The airway production of NO would change along with the expression of endothelial, neuronal, and inducible isoforms of NO synthase (eNOS, nNOS, and iNOS) in the peripheral lung tissue of COPD patients. iNOS is believed to play a critical role in the inflammatory response. iNOS is increased by inflammatory mediators and can generate tremendous amounts of NO.[28] On the one hand, the activity of iNOS in AECOPD patients is enhanced by airway inflammation.[29] On the other hand, hypoxia can induce damage to pulmonary capillaries endothelial cells and decrease eNOS activity.[30] The NO concentration produced

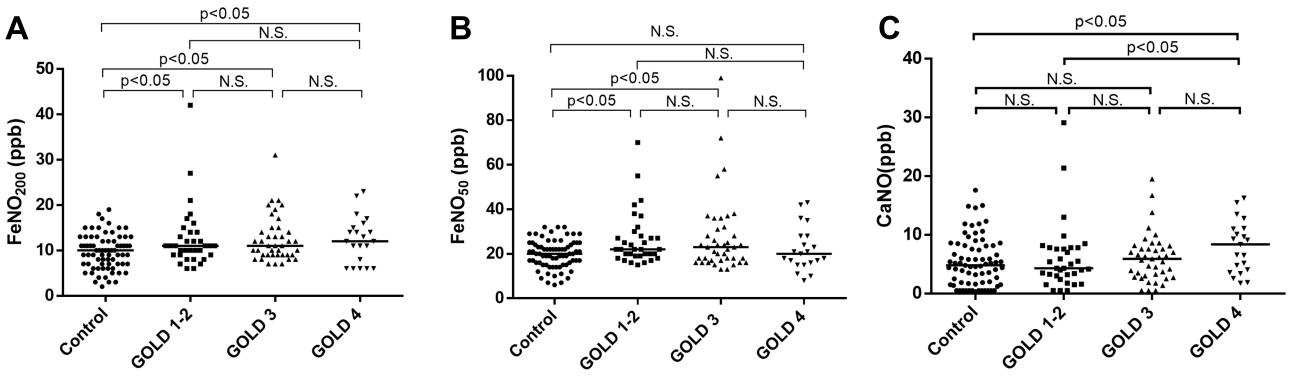

Figure 3 $FeNO_{50}$, $FeNO_{200}$ and CaNO in healthy controls and COPD patients of different severity according to the classification of the Global Initiative for Chronic Obstructive Lung Disease (GOLD). $FeNO_{50}$ was not correlated with different GOLD stages (**A**). $FeNO_{200}$ was not correlated with GOLD stages (**B**). CaNO was elevated at stage of GOLD4 (**C**).

by eNOS decreased when it diffused from alveolar capillaries to alveoli. Therefore, influenced by multiple factors, the level of NO did not change significantly in AECOPD patients. Further research is needed to understand the mechanism better.

Our findings showed that $FeNO_{50}$ of AECOPD and stable COPD patients were higher than that of healthy controls, which agreed with previous studies. Zhiyu Lu reported that only a mild elevation of $FeNO_{50}$ levels patients with stable COPD.[31] However, no significant difference was found in $FeNO_{50}$ between stable and exacerbated COPD patients. The reason may be consistent with $FeNO_{200}$ as described above, and large airway NO could be suppressed by ICS.[31] Besides, the bacterial infection is an important cause of exacerbation of COPD. There was evidence showed that bacterial infection decreased $FeNO_{50}$ levels.[32] NO levels at lower expiratory flow rates mainly indicate the bronchial inflammation; therefore, $FeNO_{50}$ cannot exactly reflect peripheral airway inflammation in COPD patients.

For AECOPD patients, there was no significant difference between $FeNO_{200}$ and CaNO after systemic corticosteroid treatment. This result may be attributed to the low initial exhaled NO values in some patients, and these patients had an inadequate response to corticosteroid treatment. By classifying patients based on the cut-off point of $FeNO_{50}$> 25, We identified that 31% of patients with higher $FeNO_{50}$ levels had a significant $FeNO_{50}$ decrease and improved CAT after corticosteroid treatment, suggesting an excellent response to corticosteroid. This finding agreed with ATS's guideline in 2011 that $FeNO_{50}$ greater than 25ppb indicates eosinophilic inflammation and high $FeNO_{50}$ in COPD patients predict a good corticosteroid response.[18,33]

Although there are no guidelines for FeNO200 and CaNO, based on existing clinical studies, the FeNO200 and CaNO values of healthy people are less than 10 ppb and 5 ppb, respectively.[18,20,35–40] Our research showed a significant decrease in CAT and $FeNO_{200}$ in patients with $FeNO_{200}$ greater than 10 ppb after corticosteroid therapy. Patients with FeNO200 below 10 ppb did not change after treatment, whereas CaNO did not change after 1 week's corticosteroid treatment neither in the high CaNO value group nor in the low CaNO value group, which is similar to the previous study.[16] The results implied that AECOPD patients with FeNO200 > 10 ppb would benefit from corticosteroid.

GOLD recommended that COPD patients with circulating eosinophils >100 cells/μL can benefit from corticosteroid application. For patients with eosinophils <100 cells/μL, corticosteroid treatment is restrained due to poor response and the increased risk of pneumonia.[41] In our study, 64% of patients had eosinophil counts greater than 100 cells/μL, similar to previous results.[42] There were 59% AECOPD patients with FeNO200 >10 ppb. However, only 31% of patients with AECOPD had FeNO50 greater than 25 ppb. Therefore, $FeNO_{50}$ cannot truly reflect COPD's inflammatory state, and only measuring FeNO50 may miss some patients who would benefit from corticosteroid application. Compared with FeNO50, FeNO200 may be a better indicator of corticosteroid therapy in COPD patients.

Conclusion

This study proved that $FeNO_{200}$ was a simpler and more patient-friendly method to directly measure the NO levels of peripheral airway/alveoli in COPD patients. In AECOPD patients with FeNO200 > 10 ppb presented a better response

to corticosteroid treatment. Moreover, it is consistent with the guidance of peripheral blood eosinophils counts. Therefore, FeNO200 can be a type 2 inflammation biomarker and a useful tool for corticosteroid treatment in COPD.

Ethics Approval and Consent to Participate

The study was approved by the ethical review board of Wuxi People's Hospital Affiliated to Nanjing Medical University. The ethics approval number was KS000024.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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