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Differential Clinical Significance of $FENO_{200}$ and CANO in Asthma, Chronic Obstructive Pulmonary Disease (COPD), and Asthma-COPD Overlap (ACO)

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Purpose: To investigate the differential clinical significance of fractional concentration of exhaled nitric oxide measured at a flow rate of 200 mL/s ($FENO_{200}$) and concentration of nitric oxide in alveolar (CANO) in asthma, chronic obstructive pulmonary disease (COPD) or asthma-COPD Overlap (ACO).

Methods: A total of 178 patients were included, with 82 patients in asthma group, 47 patients in COPD group and 49 patients in ACO group. Data for demographic data, spirometry and exhaled nitric oxide were collected for comparative analysis, correlation analysis and discriminant canonical analysis.

Results: The values of $FENO_{200}$ in asthma, COPD and ACO groups were 11.0(7.0–22.3), 8.0(6.0–11.0) and 9.0(6.5–19.5) ppb, respectively. In the asthma group, $FENO_{200}$ exhibited negative correlations with FEV_1/FVC, MMEF and MEF50. No significant correlation was observed between CANO and pulmonary function parameters. In the COPD group, both $FENO_{200}$ and CANO showed negative correlation with pulmonary function parameters including FVC, FEV_1, PEF, MMEF, MEF75, MEF50. In the ACO group, $FENO_{200}$ demonstrated no significant correlation with pulmonary function parameters, while CANO was correlated with FEV_1, PEF, MMEF and MEF50. In COPD group, ΔFEV_1 in the bronchodilator test was correlated with $FENO_{200}$. As for the ACO group, ΔFEV_1 was correlated with CANO. In the discriminant canonical analysis, four parameters including gender, age, MEF75 and $FENO_{50}$ discriminated between the three groups of asthma, COPD and ACO.

Conclusion: In asthma, COPD and ACO, $FENO_{200}$ has demonstrated a robust correlation with CANO. Elevated $FENO_{200}$ levels are predominantly indicative of pulmonary function impairment in asthma and COPD, whereas elevated CANO levels are mainly correlated with pulmonary function impairment in COPD and ACO. Compared with $FENO_{200}$ and CANO, $FENO_{50}$ may have a better discriminatory ability in distinguishing asthma, COPD and ACO.

Keywords: $FENO_{200}$, CANO, ACO, asthma, chronic obstructive pulmonary disease

Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are chronic respiratory diseases which involves small airway inflammation and dysfunction,[1–3] while Asthma-COPD Overlap (ACO) is characterized by the concurrent presence of features of both asthma and COPD.[4–6] FENO is a non-invasive, portable and convenient method, which has been extensively utilized in the diagnosis and therapeutic evaluation of asthma[7–9] and may be a potential biomarker for assessing ICS response in COPD.[10,11] Exhaled nitric oxide measured at varying expiratory flow rates is acknowledged to reflect the inflammation status within airways of different diameters.[12]

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$FENO_{50}$ is one of the most widely used biomarkers for airway inflammation, predominantly reflecting the inflammation status of large central airways but is insensitive to the changes of inflammation in peripheral airways. In recent years, CANO has garnered increased interest as a biomarker for peripheral airway inflammation, offering unique advantages over $FENO_{50}$ in the assessment of pulmonary function.[13,14] Nevertheless, to obtain the data needed to compute CANO values patients are required to perform multiple exhalations at different flow rates, which can be challenging for some patients. The study by Fan et al had pointed out that $FENO_{200}$ was associated with small airway function in COPD and might be an alternative to CANO.[15] Despite these findings, the comparative efficacy between CANO and $FENO_{200}$ remains not fully elucidated, and the clinical relevance of $FENO_{200}$ and CANO in ACO has been rarely reported. Hence, this study is an extension of the former studies and the primary objective of the present study is to investigate the differential clinical significance of $FENO_{200}$ and CANO in the context of asthma, COPD and ACO.

Methods

Patients and Study Design

This was a retrospective study conducted in Shenzhen Hospital, Southern Medical University. Data of outpatients diagnosed with asthma, COPD or ACO who presented to the respiratory department between January 2019 and January 2023 were collected. Only cases with available data for pulmonary function and exhaled nitric oxide were included. COPD was diagnosed based on the presence of non-fully reversible airflow limitation ($FEV_1/FVC<0.7$ post-bronchodilation) according to the GOLD criteria.[16] Asthma diagnoses were established following the GINA guidelines.[17] Patients fulfilling the diagnostic criteria for COPD and exhibited reversible airflow limitation (post-bronchodilation increase in $FEV_1 \geq 12\%$ and 200 mL from baseline) were diagnosed with ACO.[17] All participants were in a stable condition. The primary exclusion criteria encompassed the absence of pulmonary function data, coexistence of severe cardiovascular disease or other underlying pulmonary diseases such as lung cancer, bronchiectasis, tuberculosis, and interstitial lung disease. The flowchart of patient selection is depicted in Figure 1. A total of 178 patients were finally included, with 82 patients in asthma group, 47 patients in COPD group and 49 patients in ACO group. The study was in compliance with the Helsinki Declaration and approved by Medical Ethics Committee of Shenzhen Hospital of Southern Medical University (Ethical approval number: NYSZYYEC20240013). Informed consent was waived by the ethics committee since only pseudonymous data were analyzed and published.

Spirometry and Bronchodilator Test

Spirometry and bronchodilator test was performed using the Jaeger Masterscope System (Jaeger, Wuerzburg, Germany). The procedures were in accordance with the recommended guidelines by ATS/ERS.[18] In spirometry, subjects were instructed to perform a complete and forceful inhalation of total lung capacity (TLC) followed by a maximal exhalation of residual volume (RV). This maneuver was executed a minimum of three times to ensure reliable measurements. The best of the three spirometry values was used for analysis. For the bronchodilator test, subjects should abstain from short-acting inhaled drugs for 4 hours and from long-acting β-agonist bronchodilators or aminophylline for 12 hours prior to the test. After the baseline spirometry was performed, subjects inhaled salbutamol in four separate doses of 100 mcg. Post-bronchodilator spirometry was performed after 15 minutes and changes in forced expiratory volume in 1 second (FEV_1) were measured.[18,19]

Measurement of Exhaled Nitric Oxide

Measurement of exhaled nitric oxide was conducted based on the Sunvou-CA2122 analyzer (Sunvou, Wuxi, China). The operation process complied with the ATS/ERS standards.[20] Participants were instructed to inhale for 2–3 seconds to TLC and then exhaled immediately at a certain flow rate. The fractional concentrations of exhaled nitric oxide at flow rates of 50 mL/s and 200 mL/s were denoted as $FENO_{50}$ and $FENO_{200}$, respectively. CANO was calculated by a two-compartment model proposed by Tsoukias NM and George SC.[12,21]

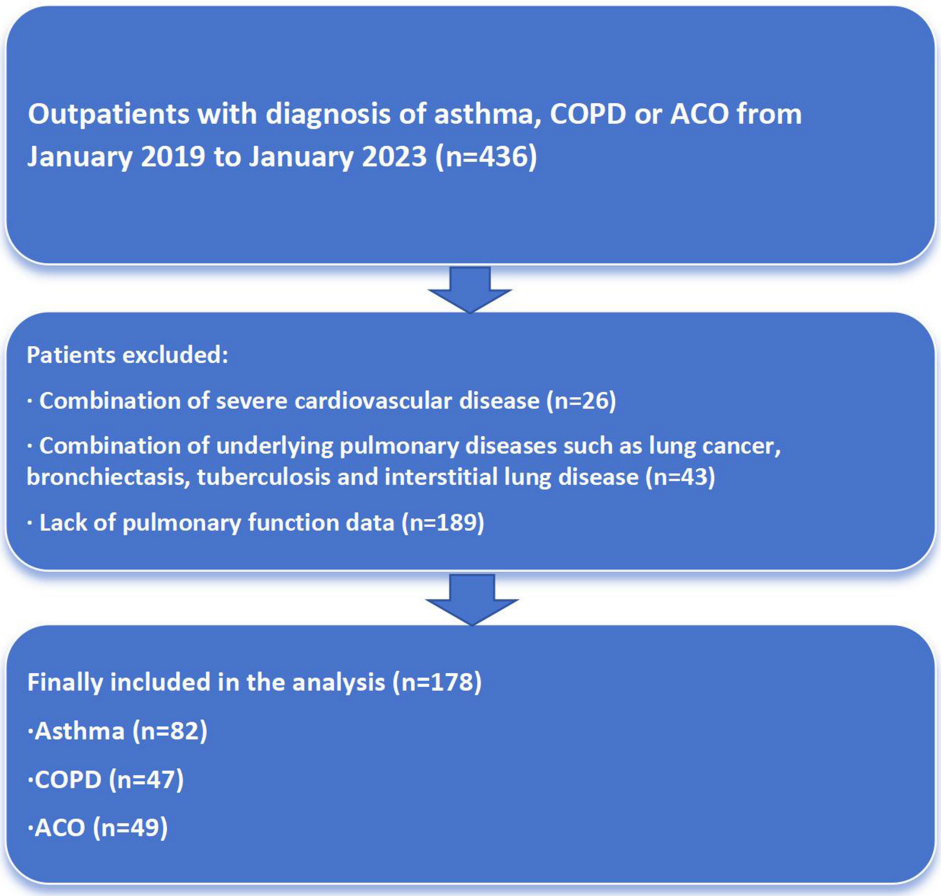

Figure 1 Flow chart showing the enrollment of patients with asthma, COPD and ACO.

Statistical Analysis

Statistical analyses were performed using IBM SPSS software, version 27.0 (SPSS, Chicago, USA). Categorical data were presented as n(%) and continuous data as mean ± standard deviation (SD) or median (interquartile range) (IQR) based on the distribution normality. Z-scores of spirometry parameters were calculated based on the GLI references.[22] For categorical data, differences between groups were compared using chi-square. For continuous data, differences between groups were compared using ANOVA for normally distributed variables and Kruskal–Wallis for non-normally distributed variables. Changes of pulmonary function in the bronchodilator test were assessed by paired *t*-test or Wilcoxon signed-rank test. The association between clinical parameters and exhaled nitric oxide levels was evaluated by Spearman's rank correlation analysis. The discriminant canonical analysis was performed using the Canonical Discriminant Function tool of SPSS. Statistical significance was defined as a p-value <0.05.

Results

Comparison of General Information and Pulmonary Function

The comparative analysis of demographic data and pulmonary function parameters among the asthma, COPD and ACO groups is detailed in Table 1. Significant intergroup differences were noted in sex distribution, age, FVC, FEV_1, FEV_1 /FVC, maximum mid-expiratory flow (MMEF) and maximum expiratory flow at 25% of vital capacity (MEF25). The asthma group demonstrated a significantly lower proportion of male subjects and younger age compared to the COPD and ACO groups. There are no significant differences observed in height, weight or BMI among the three groups. The ACO group exhibited significantly impaired pulmonary function, including FVC, FEV_1, FEV_1/FVC, MMEF and MEF25, compared to the asthma and COPD groups. A graphical representation of $FENO_{50}$, $FENO_{200}$, and CANO comparisons is

Table 1 General Information and Pulmonary Function Parameters Among Asthma, COPD and ACO Groups

Parameters	Asthma (n=82)	COPD (n=47)	ACO (n=49)	Overall P value
Males, n(%)	40(48.8)	37(78.7)	41(83.7)	<0.01[a,b]
Age, years	50.5(37.0–59.3)	66.0(59.0–72.0)	66.0(61.0–70.0)	<0.01[a,b]
Height, m	1.66(1.55–1.70)	1.63±0.01	1.66(1.59–1.71)	0.753
Weight, kg	62.6(57.1–70.2)	60.0(55.1–68.0)	64.4±11.0	0.444
BMI, kg/m^2	24.2±3.3	23.4±3.2	23.9±3.6	0.384
FVC, L	3.4±1.1	3.2±1.0	2.6(2.1–3.0)	<0.01[b,c]
FVC Z-score	0.21±1.80	0.09±1.50	−1.14±1.35	<0.01[b,c]
FEV_1, L	2.1±0.7	1.8±0.8	1.1±0.4	<0.01[a,b,c]
FEV_1 Z-score	−1.47±1.66	−1.69±1.53	−3.20±1.66	<0.01[b,c]
FEV_1/FVC, %	64.3(58.2–68.9)	56.8(43.5–66.0)	40.3(34.6–51.6)	<0.01[a,b,c]
FEV_1/FVC Z-score	−2.95±1.14	−3.27±1.27	−4.45±1.20	<0.01[b,c]
MMEF, L/s	1.0(0.6–1.6)	0.7(0.3–1.0)	0.3(0.2–0.4)	<0.01[a,b,c]
MMEF Z-score	−2.67±1.36	−2.59±0.97	−3.58±0.83	<0.01[b,c]
MEF25, L/s	0.4(0.2–0.6)	0.2(0.1–0.4)	0.1(0.1–0.2)	<0.01[a,b,c]
MEF25 Z-score	−2.36±1.31	−2.11±1.40	−3.11±1.02	<0.01[b,c]
$FENO_{50}$, ppb	26.0(17.0–54.5)	18.0(13.0–26.0)	21.0(13.0–36.5)	<0.01[a]
$FENO_{200}$, ppb	11.0(7.0–22.3)	8.0(6.0–11.0)	9.0(6.5–19.5)	<0.05[a]
CaNO, ppb	2.5(0.5–6.2)	2.8(1.8–5.0)	3.8(1.8–8.8)	0.162

Notes: Data are presented as mean±standard deviation or medians (interquartile range), unless otherwise stated. [a]P<0.05, Asthma group vs COPD group; [b]P<0.05, Asthma group vs ACO group; [c]P<0.05, COPD group vs ACO group.

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1s; MMEF, forced expiratory flow between 25 and 75%; MEF25, forced expiratory flow at 25% of the FVC; $FENO_{50}$, exhaled nitric oxide at a flow rate of 50mL/s; $FENO_{200}$, exhaled nitric oxide at a flow rate of 200mL/s; CaNO, concentration of alveolar nitric oxide.

provided in Figure 2. The values of $FENO_{200}$ in asthma, COPD and ACO groups were 11.0(7.0–22.3), 8.0(6.0–11.0) and 9.0(6.5–19.5) ppb, respectively. The values of CANO in asthma, COPD and ACO groups were 2.5(0.5–6.2), 2.8(1.8–5.0) and 3.8(1.8–8.8) ppb, respectively. The asthma group showed the highest $FENO_{50}$ and $FENO_{200}$ values, while the ACO group had the highest CANO values. However, the differences in CANO do not reach the level of significance.

Correlation Between Exhaled Nitric Oxide and Clinical Parameters

Correlation between exhaled nitric oxide and clinical parameters for each group is delineated in Tables 2–4. In the asthma group, $FENO_{50}$ exhibited significant or near-significant negative correlations with pulmonary function

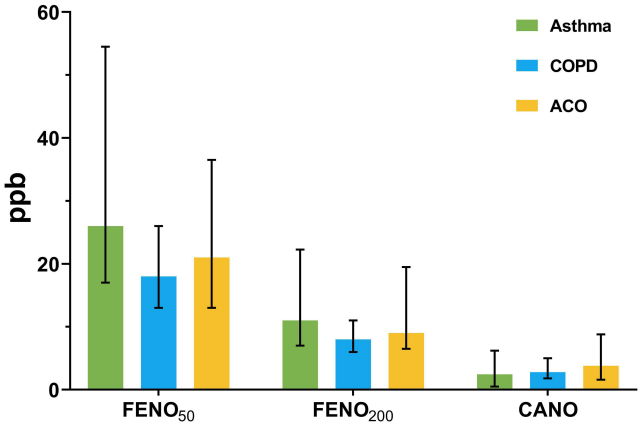

Figure 2 Values of $FENO_{50}$, $FENO_{200}$ and CANO among asthma, COPD and ACO groups showing median and interquartile range (IQR).

Table 2 Correlation Coefficients Between $FENO_{50}$, $FENO_{200}$, CANO and Clinical Parameters in Asthma Group

Parameters	$FENO_{50}$		$FENO_{200}$		CANO	
	Rho(ρ) value	P value	Rho(ρ) value	P value	Rho(ρ) value	P value
Males, n(%)	0.044	0.696	0.102	0.361	0.271	<0.05
Age, years	0.204	0.066	0.206	0.063	0.099	0.375
Height, m	0.071	0.527	0.153	0.171	0.328	<0.05
Weight, kg	−0.056	0.617	0.014	0.903	0.199	0.073
BMI, kg/m^2	−0.123	0.271	−0.048	0.667	0.079	0.478
FVC, L	−0.025	0.823	−0.025	0.820	0.140	0.211
FEV_1, L	−0.117	0.295	−0.119	0.288	0.094	0.400
FEV_1/FVC, %	−0.241	<0.05	−0.240	<0.05	−0.024	0.831
PEF, L/s	−0.094	0.399	−0.107	0.340	0.094	0.400
MMEF, L/s	−0.239	<0.05	−0.219	<0.05	0.054	0.628
MEF75, L/s	−0.122	0.276	−0.150	0.178	0.052	0.641
MEF50, L/s	−0.029	<0.05	−0.234	<0.05	0.072	0.519
MEF25, L/s	−0.215	0.054	−0.170	0.129	0.082	0.467
$FENO_{200}$, ppb	0.888	<0.01	–	–	–	–
CaNO, ppb	0.028	0.803	0.393	<0.01	–	–

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1s; PEF, peak expiratory flow; MMEF, forced expiratory flow between 25 and 75%; MEF75, forced expiratory flow at 75% of the FVC; MEF50, forced expiratory flow at 50% of the FVC; MEF25, forced expiratory flow at 25% of the FVC; $FENO_{50}$, exhaled nitric oxide at a flow rate of 50mL/s; $FENO_{200}$, exhaled nitric oxide at a flow rate of 200mL/s; CaNO, concentration of alveolar nitric oxide.

Table 3 Correlation Coefficients Between $FENO_{50}$, $FENO_{200}$, CANO and Clinical Parameters in COPD Group

Parameters	$FENO_{50}$		$FENO_{200}$		CANO	
	Rho(ρ) value	P value	Rho(ρ) value	P value	Rho(ρ) value	P value
Males, n(%)	−0.121	0.418	−0.189	0.204	−0.173	0.244
Age, years	0.160	0.283	0.125	0.401	0.006	0.967
Height, m	−0.214	0.149	−0.301	<0.05	−0.206	0.165
Weight, kg	−0.156	0.296	−0.158	0.290	−0.106	0.477
BMI, kg/m^2	0.021	0.887	0.085	0.568	0.050	0.737
FVC, L	−0.096	0.520	−0.367	<0.05	−0.510	<0.01
FEV_1, L	−0.181	0.224	−0.405	<0.01	−0.434	<0.01
FEV_1/FVC, %	−0.175	0.239	−0.256	0.083	−0.168	0.259
PEF, L/s	−0.208	0.162	−0.413	<0.01	−0.363	<0.05
MMEF, L/s	−0.223	0.137	−0.343	<0.05	−0.313	<0.05
MEF75, L/s	−0.241	0.103	−0.407	<0.01	−0.344	<0.05
MEF50, L/s	−0.296	<0.05	−0.393	<0.01	−0.284	0.056
MEF25, L/s	−0.003	0.983	−0.110	0.479	−0.231	0.132
$FENO_{200}$, ppb	0.802	<0.01	–	–	–	–
CaNO, ppb	−0.037	0.805	0.512	<0.01	–	–

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1s; PEF, peak expiratory flow; MMEF, forced expiratory flow between 25 and 75%; MEF75, forced expiratory flow at 75% of the FVC; MEF50, forced expiratory flow at 50% of the FVC; MEF25, forced expiratory flow at 25% of the FVC; $FENO_{50}$, exhaled nitric oxide at a flow rate of 50mL/s; $FENO_{200}$, exhaled nitric oxide at a flow rate of 200mL/s; CaNO, concentration of alveolar nitric oxide.

parameters such including FEV_1/FVC ($\rho = -0.241$, $P < 0.05$), MMEF ($\rho = -0.239$, $P < 0.05$), MEF50 ($\rho = -0.029$, $P < 0.05$) and MEF25 ($\rho = -0.215$, $P = 0.054$). $FENO_{200}$ is significantly correlated with FEV_1/FVC ($\rho = -0.240$, $P < 0.05$), MMEF ($\rho = -0.219$, $P < 0.05$) and MEF50 ($\rho = -0.234$, $P < 0.05$). No significant correlation was observed between CANO and pulmonary function parameters. Within the COPD group, $FENO_{50}$ was significantly correlated

Table 4 Correlation Coefficients Between $FENO_{50}$, $FENO_{200}$, CANO and Clinical Parameters in ACO Group

Parameters	$FENO_{50}$		$FENO_{200}$		CANO	
	Rho(ρ) value	P value	Rho(ρ) value	P value	Rho(ρ) value	P value
Males, n(%)	−0.037	0.800	−0.031	0.831	−0.010	0.947
Age, years	0.148	0.310	0.239	0.099	0.225	0.121
Height, m	0.143	0.326	0.050	0.734	−0.151	0.301
Weight, kg	0.086	0.558	0.008	0.957	0.140	0.336
BMI, kg/m^2	−0.025	0.863	−0.051	0.728	−0.081	0.580
FVC, L	0.131	0.370	0.028	0.848	−0.196	0.177
FEV_1, L	0.146	0.317	0.031	0.833	−0.309	<0.05
FEV_1/FVC, %	0.056	0.703	0.016	0.911	−0.173	0.235
PEF, L/s	0.073	0.620	−0.047	0.747	−0.373	<0.01
MMEF, L/s	−0.010	0.948	−0.091	0.532	−0.349	<0.05
MEF75, L/s	0.177	0.222	0.071	0.629	−0.245	0.090
MEF50, L/s	0.038	0.794	−0.053	0.720	−0.315	<0.05
MEF25, L/s	0.054	0.734	0.057	0.721	−0.167	0.290
$FENO_{200}$, ppb	0.907	<0.01	–	–	–	–
CaNO, ppb	0.355	<0.05	0.656	<0.01	–	–

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1s; PEF, peak expiratory flow; MMEF, forced expiratory flow between 25 and 75%; MEF75, forced expiratory flow at 75% of the FVC; MEF50, forced expiratory flow at 50% of the FVC; MEF25, forced expiratory flow at 25% of the FVC; $FENO_{50}$, exhaled nitric oxide at a flow rate of 50mL/s; $FENO_{200}$, exhaled nitric oxide at a flow rate of 200mL/s; CaNO, concentration of alveolar nitric oxide.

only with MEF50 ($\rho = -0.296$, $P < 0.05$), while both $FENO_{200}$ and CANO showed significant or near-significant negative correlation with pulmonary function parameters including FVC, FEV_1, PEF, MMEF, MEF75, MEF50. In the ACO group, neither $FENO_{50}$ nor $FENO_{200}$ demonstrated significant correlations with pulmonary function parameters, while CANO significantly correlated with FEV_1($\rho = -0.309$, $P < 0.05$), PEF ($\rho = -0.373$, $P < 0.05$), MMEF ($\rho = -0.349$, $P < 0.05$) and MEF50 ($\rho = -0.315$, $P < 0.05$). Additionally, across all three groups, $FENO_{200}$ exhibited significant positive correlations with both $FENO_{50}$ and CANO ($P < 0.01$).

Correlation Between Bronchodilator Reversibility and Exhaled Nitric Oxide in COPD and ACO

The bronchodilator reversibility of FEV_1 and FVC for COPD and ACO patients is presented in Table 5. Increases in FEV_1 were 4.82 ± 4.57% for the COPD group and 17.86(12.41–25.21)% for the ACO group. Corresponding increases in FVC were 2.58 ± 5.29% and 13.96(8.77–21.06)%, respectively. Significant changes were observed between pre- and post-bronchodilator FEV_1 and FVC in both COPD and ACO groups. In addition, Spearman correlation analysis was conducted to explore the potential associations between bronchodilator reversibility and exhaled nitric oxide including $FENO_{50}$, $FENO_{200}$ and CANO. In COPD group, changes in FEV_1(ΔFEV_1) was found to be significantly correlated with $FENO_{50}$($\rho = -0.320$, $P < 0.05$) and $FENO_{200}$($\rho = -0.302$, $P < 0.05$). In the ACO group, ΔFEV_1 was significantly correlated only with CANO ($\rho = 0.286$, $P < 0.05$). The results of the correlation analysis is presented in Figure 3.

Discriminant Canonical Analysis

Clinical parameters including gender, age, height, weight, FVC, FEV_1, PEF, MMEF, MEF75, MEF50, MEF25, $FENO_{50}$, $FENO_{200}$ and CANO were included into the linear discriminant analysis (LDA). The LDA based on all 14 parameters from which by stepwise exclusion 4 remained in the model is graphically represented in Figure 4. The overall prediction accuracy was 70.2%. Based on Wilks' lambda (Λ) test statistics four parameters including gender ($\Lambda = 0.457$), age ($\Lambda = 0.567$), MEF75 ($\Lambda = 0.456$) and $FENO_{50}$ ($\Lambda = 0.466$) discriminated between the three groups of asthma, COPD and ACO.

Table 5 Changes of FEV_1 and FVC in the Bronchodilation Test of COPD and ACO Patients

	COPD			ACO		
	Pre-BD	Post-BD	P value	Pre-BD	Post-BD	P value
FEV_1 (L)	1.76±0.75	1.82(1.19–2.35)	<0.01	1.11±0.42	1.22(1.02–1.56)	<0.01
FEV_1 Z-score	−1.69±1.53	−1.51±1.58	<0.01	−3.20±1.66	−2.78±1.14	<0.01
Reversibility (L)	0.07±0.07			0.07±0.17		
Reversibility (%)	4.82±4.57			17.86(12.41–25.21)		
FVC (L)	3.15±0.98	3.22±0.98	<0.01	2.56(2.12–3.03)	3.01±0.79	<0.01
FVC Z-score	0.09±1.50	0.24±1.48	<0.01	−1.14±1.35	−0.38±1.40	<0.01
Reversibility (L)	0.07±0.17			0.35(0.19–0.47)		
Reversibility (%)	2.58±5.29			13.96(8.77–21.06)		

Abbreviations: FVC, forced vital capacity; FEV_1, forced expiratory volume in 1s.

Discussion

To the best of our knowledge, this is the first report concerning the differences between $FENO_{200}$ and CANO. Both $FENO_{200}$ and CANO have the potential to serve as biomarkers for peripheral airway inflammation.[23,24] In our study, $FENO_{200}$ was observed to be positively correlated with CANO across all the groups. However, it was noteworthy that the correlation analyses yielded results that were not entirely congruent. $FENO_{200}$ demonstrated a predominant association with pulmonary function in asthma and COPD, whereas CANO showed a stronger association with pulmonary function

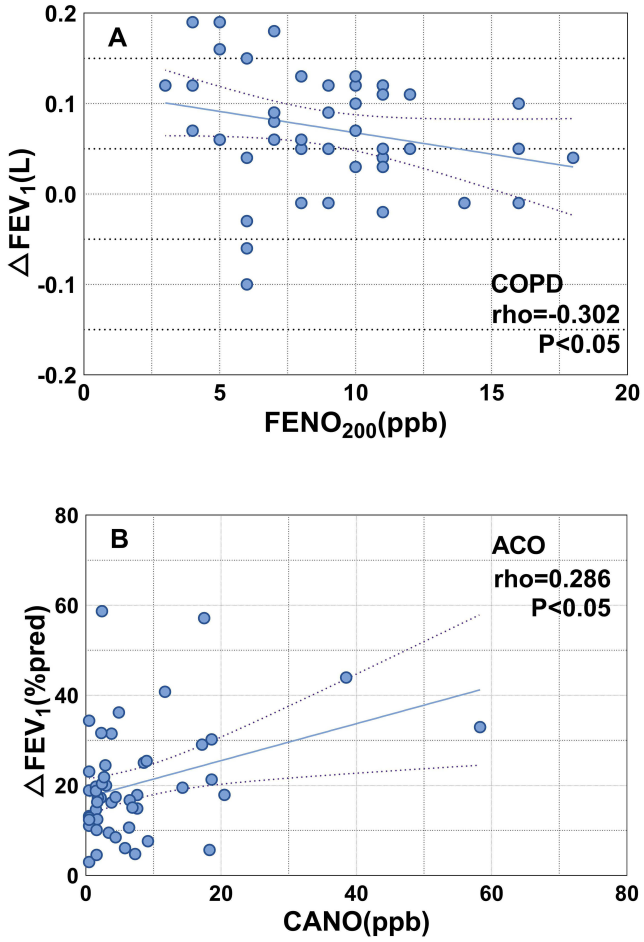

Figure 3 Correlation between ΔFEV_1 and exhaled nitric oxide in COPD and ACO. (**A**) Correlation between ΔFEV_1 (L) and $FENO_{200}$ in COPD; (**B**) Correlation between ΔFEV_1 (%pred) and CANO in ACO.

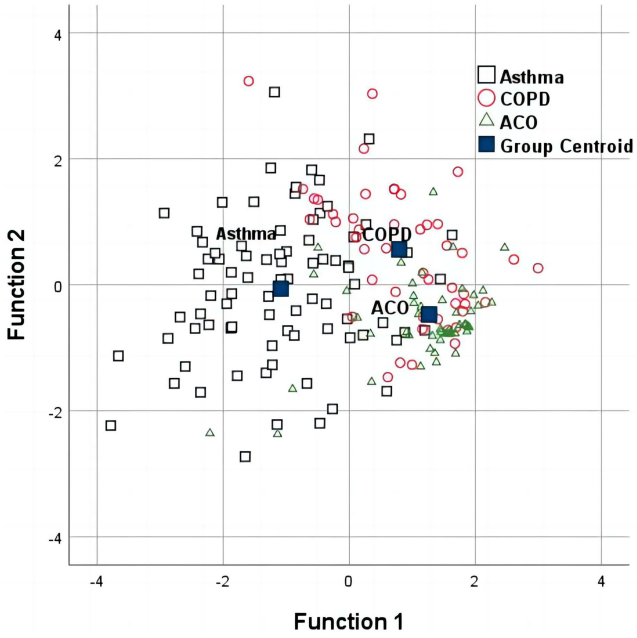

Figure 4 Linear discriminant analysis based on 14 parameters from which by stepwise exclusion 4 remained in the model (gender, age, MEF75, $FENO_{50}$), differentiating between asthma, COPD and ACO.

in COPD and ACO. Our findings suggest that $FENO_{200}$ and CANO may be differentially selected for the assessment of distinct pulmonary diseases.

FENO is recognized as a biomarker for type-2 airway inflammation, which is mainly presented in asthma and a subset of COPD patients, playing an important role in both stable condition and exacerbation.[25–27] Previous researches have suggested that patients with asthma or ACO exhibited significantly higher FENO levels than COPD,[28,29] which is consistent with our study. $FENO_{50}$ and $FENO_{200}$ were found to be highest in the asthma group, followed by the ACO group, and lowest in the COPD group (Table 1). Notably, the highest level of CANO was observed in the ACO group. Moreover, the ACO group also presented with significantly impaired pulmonary function compared to the other two groups. A possible explanation is that ACO exhibits the characteristics of both asthma and COPD, the chronic inflammatory response and oxidative stress in the distal airways may be more pronounced,[30,31] which warrant further investigation.[30]

According to the European Respiratory Society (ERS) recommendations, unlike $FENO_{50}$, there are no universally accepted reference values for CANO and $FENO_{200}$.[12] Similar to our findings, Sy Duong-Quy et al reported the highest CANO levels and the poorest pulmonary function in patients with ACO instead of asthma and COPD,[28] but the values of CANO in all three groups (4±2ppb, 3±2ppb and 6±3ppb for asthma, COPD and ACO group, respectively) were slightly higher than in our study (2.5(0.5–6.2)ppb, 2.8(1.8–5.0)ppb and 3.8(1.8–8.8)ppb for asthma, COPD and ACO group, respectively). The discrepancies observed in CANO values may be attributed to several factors, including disease severity, model of nitric oxide analyzer, expiratory flow rates and computational methods for CANO measurement.[32] Furthermore, the literature on reference values for $FENO_{200}$ is scarce. Fan et al previously reported a $FENO_{200}$ value of 11.0(9.0–15.0)ppb in stable COPD patients,[15] while a study on cough variant asthma reported a value of 17.0(10.0–24.0) ppb.[33] There is no study regarding $FENO_{200}$ values in ACO patients. Our study provides novel reference values for $FENO_{200}$ and CANO in asthma, COPD, and especially ACO.

The American Thoracic Society (ATS) has endorsed the use of $FENO_{50}$ in the diagnosis and management of asthma.[26] Elevated $FENO_{50}$ levels indicate a poor prognosis for asthma and are correlated with dysfunction of small airways,[34] aligning with our study's findings (Table 2). Besides, a similar correlation was observed in $FENO_{200}$. Our study revealed that $FENO_{200}$ was inversely associated with pulmonary function parameters including FEV_1/FVC, MMEF and MEF50. Conversely, no significant correlation was observed between CANO and pulmonary function parameters. Whether CANO is associated with impairment of pulmonary function in asthma remains a contentious issue.[35] While a study on refractory asthma in pediatric populations reported a negative association between CANO and MMEF, in

another study on adult asthma, no significant correlation was observed between CANO and either MMEF or MEF50.[36,37] The observed differences were likely attributable to the patient characteristics, disease severity and the treatment modalities utilized. Additionally, a recent study by Bai et al had shown that $FENO_{200}$ exhibited a more robust correlation with small airway function and was potentially more useful than CANO in diagnosing cough variant asthma.[33] Our results suggest that $FENO_{200}$ may offer advantages over CANO for the assessment of pulmonary function in asthma.

It is believed that airway inflammation in COPD is primarily driven by type-1 immune response.[25] However, type-2 inflammatory immunity is also present in 15–37% of COPD patients, indicating its role in COPD pathogenesis.[38,39] Compared with asthma, influenced by factors including smoking habits, phenotypic heterogeneity and disease severity, the role of FENO in COPD has not been well defined.[40] Previous study suggested that elevated FENO levels is associated with increased symptoms, impaired pulmonary function and frequent exacerbations in COPD.[34] In our study, both $FENO_{200}$ and CANO demonstrated significant inverse correlation with pulmonary function parameters, which indicates that $FENO_{200}$ and CANO are efficient in reflecting the inflammatory levels and pulmonary function in COPD.

ACO is characterized by an incompletely reversible airway obstruction accompanied by symptoms or signs of increased reversibility of obstruction.[32] Former studies have shown that ACO patients are more symptomatic and exhibit higher frequency of exacerbations and a worse quality of life.[41,42] Without appropriate treatment, patients with ACO may have worse prognosis than those with asthma of COPD alone. A study by Deng et al on ACO had found that there was no significant correlation between $FENO_{50}$ and pulmonary function parameters including FEV_1 and FEV_1/FVC, which was in agreement with our study.[43] As mentioned in the introduction, there have been very few studies on the clinical significance of $FENO_{200}$ and CANO in patients with ACO. In the current study, we observed that pulmonary function parameters such as FEV_1, PEF, MMEF and MEF50 were only correlated with CANO but not $FENO_{200}$. Since this is the first report comparing $FENO_{200}$ and CANO, further in-depth studies are required to understand the mechanisms behind these discrepancies.

In COPD and ACO groups, we further investigated the correlation between bronchodilator reversibility and exhaled nitric oxide. It is well established in the literature that exhaled nitric oxide is associated with eosinophilic airway inflammation and the response to glucocorticoids in asthma and ACO.[44,45] Our study showed that ACO patients with elevated CANO levels exhibited better bronchodilator reversibility. In contrast, we found that elevated $FENO_{200}$ levels was associated with worse bronchodilator reversibility in COPD. It had been previously reported in a few studies that a minor degree of airway obstruction in COPD could result in a reduced ΔFEV_1% during bronchodilator test and an increase in FENO values.[23,46–48] Given that the majority of COPD patients enrolled in our study were mainly classified within GOLD I–II stages, this might provide a plausible explanation for the results.

Notably, the canonical discriminant analysis revealed that age exerted the most discriminative power, succeeded by $FENO_{50}$, gender and MEF75. $FENO_{200}$ and CANO were excluded from the final model. This implies that, although $FENO_{200}$ and CANO demonstrate significant correlation with spirometry parameters as well as bronchodilator reversibility, $FENO_{50}$ may have a better discriminatory ability in distinguishing asthma, COPD and ACO.

Some limitations need to be noted regarding the present study. First, as this was a retrospective study, the differences in the inherent disease characteristics might have influenced the results. Second, since previous study has indicated that plethysmography measurements such as sRawtot and sRaweff provide additional value in the evaluation of peripheral airway dysfunction,[49] the plethysmography measurements should be incorporated in the future studies. Finally, the underlying mechanisms that contribute to the observed correlation between $FENO_{200}$ and small airways necessitate further exploration and elucidation.

Conclusion

In asthma, COPD and ACO, $FENO_{200}$ has demonstrated a robust correlation with CANO. Elevated $FENO_{200}$ levels are predominantly indicative of pulmonary function impairment in asthma and COPD, whereas elevated CANO levels are mainly correlated with pulmonary function impairment in COPD and ACO. Compared with $FENO_{200}$ and CANO, $FENO_{50}$ may have a better discriminatory ability in distinguishing asthma, COPD and ACO.

Ethical Approval

This retrospective cohort study was approved by the Medical Ethics Committee of Shenzhen Hospital of Southern Medical University (Ethical approval number: NYSZYYEC20240013).

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Disclosure

The authors report no conflicts of interest in this work.

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