

Differential Clinical Significance of FENO₂₀₀ 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 (FENO₂₀₀) 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 FENO₂₀₀ 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, FENO₂₀₀ exhibited negative correlations with FEV₁/FVC, MMEF and MEF50. No significant correlation was observed between CANO and pulmonary function parameters. In the COPD group, both FENO₂₀₀ and CANO showed negative correlation with pulmonary function parameters including FVC, FEV₁, PEF, MMEF, MEF75, MEF50. In the ACO group, FENO₂₀₀ demonstrated no significant correlation with pulmonary function parameters, while CANO was correlated with FEV₁, PEF, MMEF and MEF50. In COPD group, ΔFEV₁ in the bronchodilator test was correlated with FENO₂₀₀. As for the ACO group, ΔFEV₁ was correlated with CANO. In the discriminant canonical analysis, four parameters including gender, age, MEF75 and FENO₅₀ discriminated between the three groups of asthma, COPD and ACO.

Conclusion: In asthma, COPD and ACO, FENO₂₀₀ has demonstrated a robust correlation with CANO. Elevated FENO₂₀₀ 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 FENO₂₀₀ and CANO, FENO₅₀ may have a better discriminatory ability in distinguishing asthma, COPD and ACO.

Keywords: FENO₂₀₀, 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

FENO₅₀ 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 FENO₅₀ 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 FENO₂₀₀ 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 FENO₂₀₀ remains not fully elucidated, and the clinical relevance of FENO₂₀₀ 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 FENO₂₀₀ 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 (FEV₁/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 FEV₁≥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: NYSZYEC20240013). 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 (FEV₁) 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 FENO₅₀ and FENO₂₀₀, respectively. CANO was calculated by a two-compartment model proposed by Tsoukias NM and George SC.^{12,21}

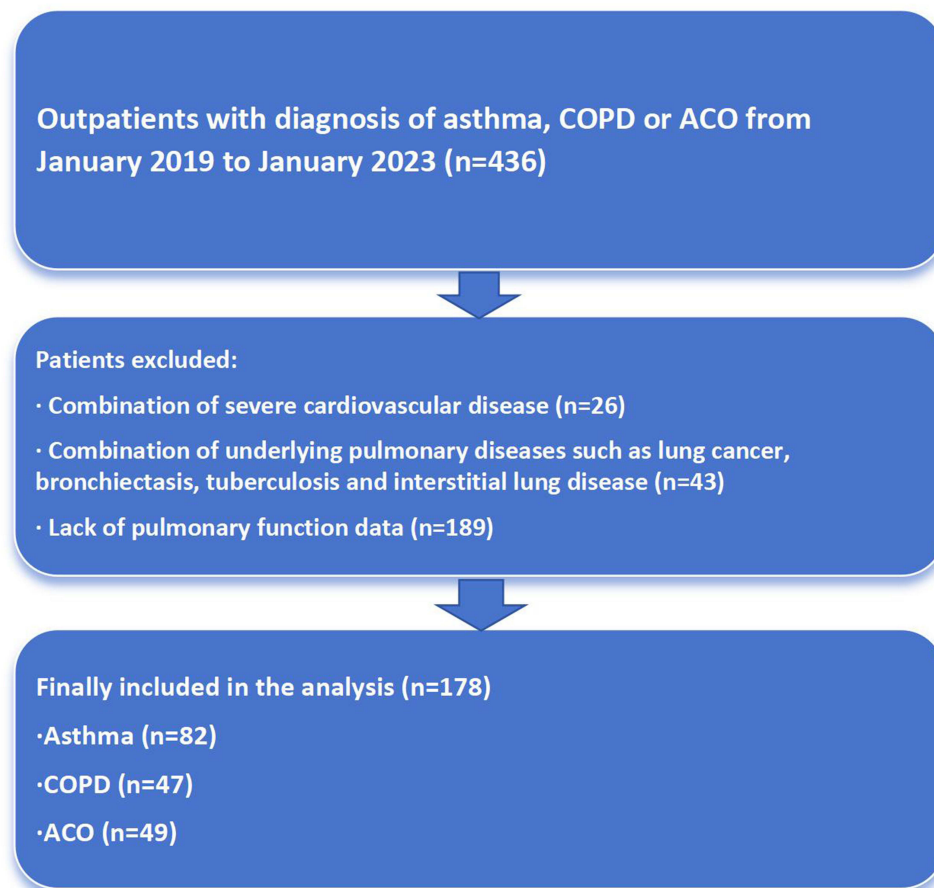


Figure 1 Flow chart showing the enrollment of patients with asthma, COPD and ACO.

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 \pm 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, FEV₁, FEV₁/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, FEV₁, FEV₁/FVC, MMEF and MEF25, compared to the asthma and COPD groups. A graphical representation of FENO₅₀, FENO₂₀₀, 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. ^aP<0.05, Asthma group vs COPD group; ^bP<0.05, Asthma group vs ACO group; ^cP<0.05, COPD group vs ACO group.

Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1s; MMEF, forced expiratory flow between 25 and 75%; MEF25, forced expiratory flow at 25% of the FVC; FENO₅₀, exhaled nitric oxide at a flow rate of 50mL/s; FENO₂₀₀, exhaled nitric oxide at a flow rate of 200mL/s; CaNO, concentration of alveolar nitric oxide.

provided in Figure 2. The values of FENO₂₀₀ 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 FENO₅₀ and FENO₂₀₀ 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, FENO₅₀ exhibited significant or near-significant negative correlations with pulmonary function

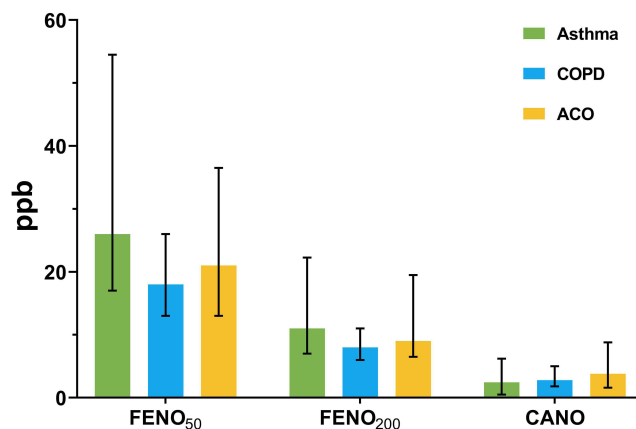


Figure 2 Values of FENO₅₀, FENO₂₀₀ and CANO among asthma, COPD and ACO groups showing median and interquartile range (IQR).

Table 2 Correlation Coefficients Between FENO₅₀, FENO₂₀₀, 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; FEV₁, 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; FENO₅₀, exhaled nitric oxide at a flow rate of 50mL/s; FENO₂₀₀, exhaled nitric oxide at a flow rate of 200mL/s; CaNO, concentration of alveolar nitric oxide.

Table 3 Correlation Coefficients Between FENO₅₀, FENO₂₀₀, 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; FEV₁, 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; FENO₅₀, exhaled nitric oxide at a flow rate of 50mL/s; FENO₂₀₀, exhaled nitric oxide at a flow rate of 200mL/s; CaNO, concentration of alveolar nitric oxide.

parameters such including FEV₁/FVC ($\rho = -0.241$, $P < 0.05$), MMEF ($\rho = -0.239$, $P < 0.05$), MEF50 ($\rho = -0.029$, $P < 0.05$) and MEF25 ($\rho = -0.215$, $P = 0.054$). FENO₂₀₀ is significantly correlated with FEV₁/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, FENO₅₀ was significantly correlated

Table 4 Correlation Coefficients Between FENO₅₀, FENO₂₀₀, 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; FEV₁, 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; FENO₅₀, exhaled nitric oxide at a flow rate of 50mL/s; FENO₂₀₀, 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 FENO₂₀₀ and CANO showed significant or near-significant negative correlation with pulmonary function parameters including FVC, FEV₁, PEF, MMEF, MEF75, MEF50. In the ACO group, neither FENO₅₀ nor FENO₂₀₀ demonstrated significant correlations with pulmonary function parameters, while CANO significantly correlated with FEV₁ ($\rho = -0.309$, $P < 0.05$), 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, FENO₂₀₀ exhibited significant positive correlations with both FENO₅₀ and CANO ($P < 0.01$).

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

The bronchodilator reversibility of FEV₁ and FVC for COPD and ACO patients is presented in Table 5. Increases in FEV₁ were $4.82 \pm 4.57\%$ for the COPD group and $17.86(12.41-25.21)\%$ for the ACO group. Corresponding increases in FVC were $2.58 \pm 5.29\%$ and $13.96(8.77-21.06)\%$, respectively. Significant changes were observed between pre- and post-bronchodilator FEV₁ 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 FENO₅₀, FENO₂₀₀ and CANO. In COPD group, changes in FEV₁ (Δ FEV₁) was found to be significantly correlated with FENO₅₀ ($\rho = -0.320$, $P < 0.05$) and FENO₂₀₀ ($\rho = -0.302$, $P < 0.05$). In the ACO group, Δ FEV₁ 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, FEV₁, PEF, MMEF, MEF75, MEF50, MEF25, FENO₅₀, FENO₂₀₀ 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 FENO₅₀ ($\Lambda = 0.466$) discriminated between the three groups of asthma, COPD and ACO.

Table 5 Changes of FEV₁ 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; FEV₁, forced expiratory volume in 1s.

Discussion

To the best of our knowledge, this is the first report concerning the differences between FENO₂₀₀ and CANO. Both FENO₂₀₀ and CANO have the potential to serve as biomarkers for peripheral airway inflammation.^{23,24} In our study, FENO₂₀₀ 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. FENO₂₀₀ demonstrated a predominant association with pulmonary function in asthma and COPD, whereas CANO showed a stronger association with pulmonary function

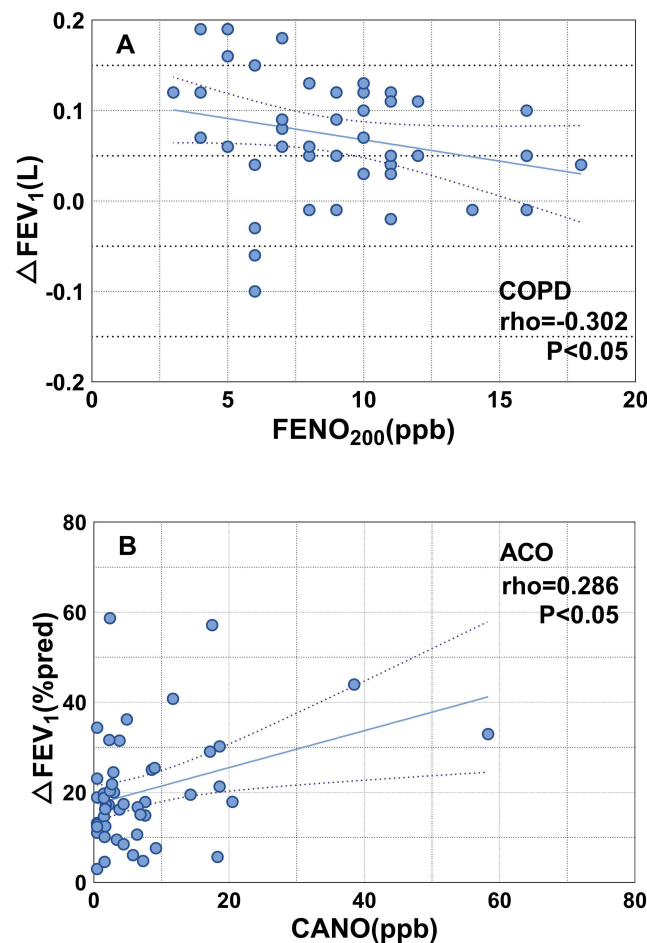


Figure 3 Correlation between ΔFEV₁ and exhaled nitric oxide in COPD and ACO. **(A)** Correlation between ΔFEV₁ (L) and FENO₂₀₀ in COPD; **(B)** Correlation between ΔFEV₁ (%pred) and CANO in ACO.

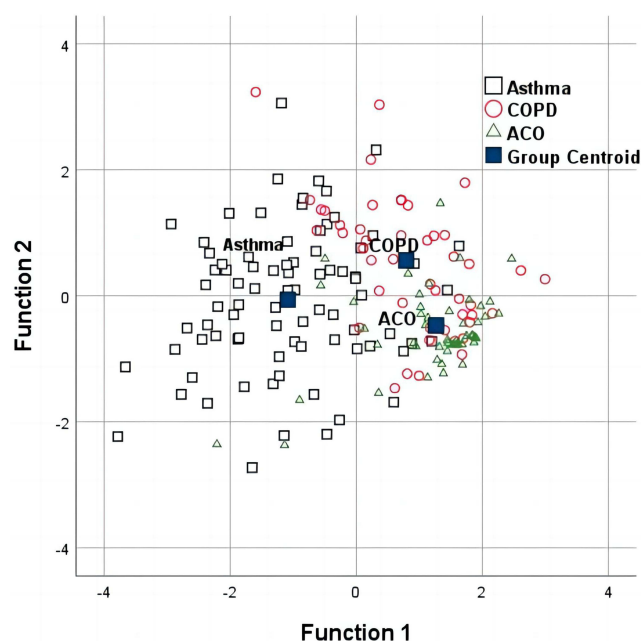


Figure 4 Linear discriminant analysis based on 14 parameters from which by stepwise exclusion 4 remained in the model (gender, age, MEF75, FENO₅₀), differentiating between asthma, COPD and ACO.

in COPD and ACO. Our findings suggest that FENO₂₀₀ 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. FENO₅₀ and FENO₂₀₀ 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 FENO₅₀, there are no universally accepted reference values for CANO and FENO₂₀₀.¹² 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 FENO₂₀₀ is scarce. Fan et al previously reported a FENO₂₀₀ 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 FENO₂₀₀ values in ACO patients. Our study provides novel reference values for FENO₂₀₀ and CANO in asthma, COPD, and especially ACO.

The American Thoracic Society (ATS) has endorsed the use of FENO₅₀ in the diagnosis and management of asthma.²⁶ Elevated FENO₅₀ 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 FENO₂₀₀. Our study revealed that FENO₂₀₀ was inversely associated with pulmonary function parameters including FEV₁/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 FENO₂₀₀ 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 FENO₂₀₀ 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 FENO₂₀₀ and CANO demonstrated significant inverse correlation with pulmonary function parameters, which indicates that FENO₂₀₀ 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 or COPD alone. A study by Deng et al on ACO had found that there was no significant correlation between FENO₅₀ and pulmonary function parameters including FEV₁ and FEV₁/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 FENO₂₀₀ and CANO in patients with ACO. In the current study, we observed that pulmonary function parameters such as FEV₁, PEF, MMEF and MEF50 were only correlated with CANO but not FENO₂₀₀. Since this is the first report comparing FENO₂₀₀ 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 FENO₂₀₀ 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 Δ FEV₁% 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 FENO₅₀, gender and MEF75. FENO₂₀₀ and CANO were excluded from the final model. This implies that, although FENO₂₀₀ and CANO demonstrate significant correlation with spirometry parameters as well as bronchodilator reversibility, FENO₅₀ 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 sRaw_{tot} and sRaw_{eff} 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 FENO₂₀₀ and small airways necessitate further exploration and elucidation.

Conclusion

In asthma, COPD and ACO, FENO₂₀₀ has demonstrated a robust correlation with CANO. Elevated FENO₂₀₀ 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 FENO₂₀₀ and CANO, FENO₅₀ 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: NYSZYEC20240013).

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Disclosure

The authors report no conflicts of interest in this work.

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