Heliyon 10 (2024) e36124

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Small airway inflammation in atypical asthma

Junjie Han [1], Li Li [1], Ying Gong [1], Juan Song [1], Yichun Zhu [1], Cuicui Chen [1], Lin Shi [1], Jian Wang [1], Yuanlin Song [**], Jun She [*]

Department of Pulmonary and Critical Care Medicine, Zhongshan Hospital, Fudan University, Shanghai, China

ARTICLE INFO

Keywords:
Classic asthma
Cough variant asthma
Chest tightness variant asthma
Small airway inflammation
Small airway dysfunction

ABSTRACT

Background: Less attention has been paid to the pathophysiological changes in atypical asthma such as cough variant asthma (CVA) and chest tightness variant asthma (CTVA). The obstruction of large and small airways is the important component in the development of asthma. We investigated whether small airway inflammation (SAI) induced small airway dysfunction (SAD) in these atypical asthmatics.

Methods: Six hundred and eighty-six patients were enrolled and analyzed in the study. The partitioned airway inflammation was assessed by fractional exhaled nitric oxide (FeNO), such as FnNO, $FeNO_{50}$, $FeNO_{200}$, and calculated alveolar fraction of exhaled NO ($CaNO_{dual}$). Correlations between exhaled NOs and SAD-related variables were assessed, whereas cell classification was evaluated by Spearman's rank tests. Classic asthma, CVA, and CTVA about potential risk were conducted using binary logistic regression models.

Results: The whole airway inflammation increased in classic and atypical asthma than controls, whereas the central and peripheral airway inflammation in the CVA and CTVA groups increased compared with the classic asthma group. Smoking exposure was found to increase the central and peripheral airway inflammation in patients with asthma. The exhaled NO of $FeNO_{50}$ and $FeNO_{200}$ was associated with SAD in classic asthma, but not in CVA and CTVA. $FeNO_{200}$ was the main risk (adjusted odds ratio [OR], 1.591; 95 % CI, 1.121–2.259; $P = .009$) in classic asthma and (adjusted OR, 1.456; 95 % CI, 1.247–1.700; $P = .000$) in CVA. The blood eosinophil levels were correlated with $FeNO_{50}$ and $FeNO_{200}$ in classic asthma and atypical asthma.

Conclusion: More severe inflammatory process was present in central and peripheral airways in CVA and CTVA, which might reflect a pre-asthmatic state. SAI was the predominant risk factor in the development of asthma before SAD.

Abbreviations: AUC, area under the curve; BMI, body mass index; CaNO, concentration of alveolar NO; CTVA, chest tightness variant asthma; CVA, cough variant asthma; FeNO, fractional exhaled nitric oxide; FEF, forced expiratory flow; FEV_1, forced expiratory volume in 1 s; FRC, functional residual capacity; FVC, forced vital capacity; GINA, Global Initiative for Asthma guideline; HRCT, high-resolution CT; IgE, immunoglobulin E; NO, nitric oxide; PFT, pulmonary function test; RV, residual volume; SAD, small airway dysfunction; SAI, small airway inflammation; TLC, total lung capacity.

* Corresponding author. Department of Pulmonary Medicine, Zhongshan Hospital, Fudan University, 180 Feng Lin Road, Shanghai, 200032, China.

** Corresponding author. Department of Pulmonary Medicine, Zhongshan Hospital, Fudan University, 180 Feng Lin Road, Shanghai, 200032, China.

E-mail addresses: song.yuanlin@zs-hospital.sh.cn (Y. Song), she.jun@zs-hospital.sh.cn (J. She).

[1] Junjie Han, Li Li, Ying Gong, Juan Song, Yichun Zhu, Cuicui Chen, Lin Shi, Jian Wang contributed equally.

https://doi.org/10.1016/j.heliyon.2024.e36124
Received 28 September 2023; Received in revised form 6 August 2024; Accepted 9 August 2024
Available online 15 August 2024

1. Introduction

Asthma is a heterogeneous disease, characterized by chronic airway inflammation and variable airway obstruction such as wheezing, breathlessness, chest tightness, and coughing [1]. It is a common respiratory disease affecting all age groups imposing a significant health burden worldwide [2]. The obstruction of large and small airways is an important component of asthma pathophysiology [3]. The accumulating evidence indicates that abnormal small airways (diameter of $\leq$2 mm) play a crucial role in the disease processes of asthma. Indeed, asthma control remains inadequate in 50 %–60 % of patients despite its guideline-based management, and untreated small airway dysfunction (SAD) has been proposed to be a contributing factor for this inadequate control [4,5]. Currently, several tests are available to measure SAD, but there is no gold standard to assess the SAD [6,7]. Different techniques measure various aspects of damage to small airways. Inflammation initially affects SAD followed by remodeling changes contributing to the clinical manifestations of asthma [8,9]. Therefore, the investigation small airway inflammation (SAI) may prove to be one of the accurate means to evaluating the early pathophysiology state of asthma.

Atypical asthma, such as cough variant asthma (CVA) [10] and chest tightness variant asthma (CTVA) [11,12], has been recognized as a precursor of typical asthma or a pre-asthmatic state. Traditional explanations include differences in the airway sensitivity or small airway function [13]. Although SAI is commonly present in patients with classic asthma, SAI in atypical asthma (CVA and CTVA) has not been fully understood. In addition, approximately 25 %–30 % of individuals with asthma are smokers [14]. It is well documented that smoking in asthma increases the morbidity and disease severity [15]; however, there is limited information on the effects of cigarette smoking on SAI in atypical asthma. Apart from smoking, Indoor environment factors have a significant impact on human being as most individuals spend around 90 % of their time indoors, either at home or work [16]. These indoor air populations can be generated within homes or buildings as a result of tenant activities such as cooking, using electronic gadgets, purchasing consumer goods, or emitting from building materials [17]. Inside buildings, harmful pollutants such carbon monoxide, volatile organic compounds, particulate matter, microplastics, allergens, aerosol, biological contaminants and other irritants can indeed contribute to the onset and worsening of asthma symptoms [18–22]. We postulated that SAI-induced SAD may be involved in these atypical asthmatic diseases (CVA and CTVA). However, it is difficult to assess the SAI by pathological examination or bronchoalveolar lavage, which is the main source of information [23]. Nitric oxide (NO), biosynthesized from L-arginine and oxygen by the enzyme NO synthase endogenously, is now widely used to determine the airway inflammation. The fractional concentration of exhaled NO at different flow rates is used to access the partitioned airway inflammation of asthma as a noninvasive and reproducible method [24]. Although fractional exhaled nitric oxide ($FeNO_{50}$) (flow rate of 50 mL/s) is a known marker of large airway inflammation [25], $FeNO_{200}$ (flow rate of 200 mL/s) is proportional to the concentration of alveolar NO (CaNO). By measuring the exhaled NO at these two different flow rates, the inflammation of central and peripheral airways can be determined [26,27].

The study aims to investigate the partitioned airway inflammation in atypical asthma phenotypes (CVA and CTVA) was measured using fractional exhaled NO (FeNO) including FnNO (the nasal NO at 10 mL/s), $FeNO_{50}$, $FeNO_{200}$, and calculated CaNO. The effects of cigarette smoking on SAI were also determined. We evaluated the correlation between FeNO and SAD, and the risk for developing asthma. We hypothesize that SAI-induced SAD is prevalent in atypical asthma and that smoking exacerbates this inflammation.

2. Methods

2.1. Study design and patients

The cross-sectional study was conducted at Zhongshan Hospital, Fudan University, Shanghai, China. A total of 2012 patients with respiratory symptoms who were referred to the Department of Pulmonary and Critical Care Medicine were screened from April 2021 to December 2022 for the presence of classic asthma, CVA, and CTVA.

Classic asthma was defined as respiratory symptoms such as wheezing, shortness of breath, chest tightness, and cough using pulmonary function and bronchodilator reversibility test [increase in forced expiratory volume in 1 s (FEV_1) of >12 % and >200 mL from baseline, 10–15 min after 200–400 mg salbutamol {Ventolin; GlaxoSmithKline}]. If the spirometry results did not support the suspicion of asthma, the patients were subjected to peak expiratory flow (PEF) monitoring twice daily over 2 weeks. The Global Initiative for Asthma (GINA) guidelines define asthma as an average daily diurnal PEF variability of >20 % or twice a day readings of >10 % variability at each reading from baseline after 4 weeks of anti-inflammatory treatment or bronchial challenge test (fall in FEV_1 of >20 % from baseline) [1]. The CVA is defined as persistent cough as the principal or only symptom with airway hyperresponsiveness and variability in lung function. For CTVA, chest tightness was considered as the sole symptom besides meeting at least one of the following criteria: (1) an increase of >12 % and >200 mL in FEV_1 after inhaling salbutamol, (2) positive result of bronchial provocation test, (3) a weekly variability in the diurnal PEF of >10 %, and (4) a marked clinical improvement in response to asthma therapy [11,28]. For the control group, healthy individuals with no history of smoking or respiratory disease were recruited in the study.

Patients with pneumonia, lung shadow or mass after chest CT scan, and chronic obstructive pulmonary disease (COPD) defined as having a postbronchodilator FEV_1 to forced vital capacity (FVC) ratio of <0.70 according to the Global Initiative for Chronic Obstructive Lung Disease guidelines [1,29], asthma–COPD overlap, allergic broncho-pulmonary aspergillosis, refractory asthma, and age <18 years were excluded.

The study was approved (B2018-010R) by the Ethics Committee of Zhongshan Hospital, Fudan University and was conducted in accordance with the Declaration of Helsinki. All patients provided signed informed consent forms.

2.2. Exhaled NO measurement

All patients were examined for exhaled NO at 3 flow rates including FnNO (the nasal NO at 10 mL/s), $FeNO_{50}$ (exhaled NO at 50 mL/s), and $FeNO_{200}$ (exhaled NO at 200 mL/s) to assess the partitioned airway inflammation [30], such as upper airway, central airway, peripheral airway/alveolar using the electrochemical Nano Coulomb Breath Analyzer (Sunvou-CA2122, Wuxi, China). The methods were according to the American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations [25,31]. Eating, smoking, drinking, strenuous exercise, or pulmonary function tests (PFTs) were prohibited 1 h before the examination.

The concentration of alveolar fraction of exhaled NO (CaNO) is the concentration of NO in the gas phase of the peripheral airway or alveolar region. On the basis of the ERS technical standard recommendations, a linear model requires at least 3 flow rates of 100 mL/s or more to calculate the CaNO, and the highest flow rate needs to reach 350 mL/s or 400 mL/s [25]. CaNO was calculated based on the same linear model using formula: FeNO = CaNO + JawNO/VE.

It is reported that approximately half of the patients with chronic airway disease do not complete the measurement at the flow rate of 350 mL/s [27], and it would influence the analysis in our study. To improve the measurement success, a simplified method was developed by using $FeNO_{50}$ and $FeNO_{200}$ [27,32]. The calculated CaNO was based on dual-flow: $FeNO = CaNO_{dual} + JawNO/VE + f$; where, FeNO was fractional concentration of exhaled NO in the gas phase parts per billion, JawNO is the total flux of NO in the conducting airway compartment ($nL.s^{-1}$), VE was exhalation flow rate (mL/s), and f was the correction factor by comparison with multi-flow CaNO literature.

2.3. Data collection

Data on demographic characteristics including age, gender, weight, height, body mass index (BMI), and smoking status were obtained. Pulmonary function test (PFT) was examined by spirometry (Jaeger, Master Screen Pulmonary Function Test, Germany). The SAD-related variables such as forced expiratory flow $(FEF)_{25\text{-}75}$ % predicted, FEF_{50} % predicted, FEF_{75} % predicted, functional residual capacity (FRC), and residual volume (RV)/total lung capacity (TLC) were collected. For the smoking group, smoking was defined as more than 1 cigarette per day for 1 year. Duration of smoking per year and per day or cessation of smoking was recorded. White cell counts and immunoglobulin E (IgE) levels in the blood were also measured. High-resolution computed tomography (HRCT) was used to exclude lung infection and lung shadow or mass.

2.4. Statistical analysis

All statistical analyses for patients' characteristics were reported as mean (standard deviation, SD) or as percentage in groups. Parameters such as age, gender, height, weight, BMI, smoking status, pulmonary function, and concentrations of FnNO, $FeNO_{50}$, $FeNO_{200}$, and $CaNO_{dual}$ in classic asthma, CVA, CTVA, and healthy control groups were analyzed using one-way analysis of variance test. Spearman's rank test was used to study the correlation test of related variables. Correlations between the exhaled NO of FnNO, $FeNO_{50}$, $FeNO_{200}$, $CaNO_{dual}$ and SAD-related variables such as $FEF_{25\text{-}75}$ % predicted, FEF_{50}, FEF_{50} % predicted, FEF_{75}, FEF_{75} % predicted, FRC% predicted, RV% predicted, TLC% predicted, RV/TLC% predicted were evaluated using Spearman's rank tests [33]. And the correlations between FnNO, $FeNO_{50}$, $FeNO_{200}$, $CaNO_{dual}$, and white cell counts including the percentage and the absolute count of neutrophils, lymphocyte, monocyte, eosinophils, basophils, and IgE were evaluated using Spearman's rank tests.

Logistic regression models were used for factor analysis, prediction and discrimination. The variable (Y) is the probability of an event occurring. While for poisson regression, the variable (Y) is a count data, e.g. the times of event occurred during a certain period. Calculations of odds ratio (OR) and 95 % confidence interval (CI) values for classic asthma, CVA, CTVA in relation to potential risk were calculated with binary logistic regression models [34]. The covariates included FnNO, $FeNO_{50}$, $FeNO_{200}$, $CaNO_{dual}$, FEV_1% predicted, FVC% predicted, FEV_1/FVC, FRC% predicted, RV% predicted, RV/TLC, RV/TLC% predicted, $FEF_{25\text{-}75}$, $FEF_{25\text{-}75}$ % predicted, FEF_{50}, FEF_{50} % predicted, FEF_{75}, FEF_{75} % predicted, age, height, weight, and BMI. Finally, the receiver operating characteristic (ROC) curves were also drawn to find the cut-off value of $FeNO_{200}$ and $CaNO_{dual}$. Sensitivity and specificity of $FeNO_{200}$ and $CaNO_{dual}$ were compared using area under the curve (AUC).

With a two-sided $\alpha = .05$ and a power of 90 %, we calculated a requirement for 208 patients per group, or a total of 409 patients. All hypothesis tests were two-sided, and a P-value of 0.05 was deemed significant. Statistical analyses were conducted with validated software packages (SAS 9.4, SAS Institute Inc and SPSS26, IBM).

3. Results

The study aims to investigate segmented airway inflammation in different types of atypical asthma, using the exhaled NO measures. It also examined the effects of cigarette smoking on segmented airway inflammation, and the correlation between FeNO and SAD, as well as the risk for the development of asthma.

3.1. Baseline characteristics

A total of 686 patients were enrolled in the analysis and grouped into classic asthma (n = 227), CVA (n = 208), CTVA (n = 31), and control (n = 220) groups (**Fig. 1**). The baseline characteristics were presented in **Table 1**. The demographics such as age, gender, height, weight, and BMI were comparable across the patients with classic asthma, CVA, CTVA, and healthy controls. The PFT

parameters such as FVC, FVC% predicted, FEV_1, FEV_1% predicted, FEV_1/FVC were similar, whereas FEF_{25-75} % predicted, FEF_{50} % predicted, FEF_{75} % predicted reduced in the classic asthma group compared with the CVA, CTVA, and control groups ($p < .01$).

3.2. Exhaled NO increased in classic and atypical asthma

The exhaled NO as measured by FnNO, $FeNO_{50}$, $FeNO_{200}$, and calculated $CaNO_{dual}$ was significantly higher in the classic asthma, CVA, and CTVA groups than control groups ($P < .01$) (**Fig. 2A**), indicating spread of inflammation across upper airway, central airway, and peripheral airway in both classical and atypical asthma. Interestingly, the tendency of central and peripheral airway inflammation was increased in the atypical asthma (CVA and CTVA) groups compared with the classic asthma group. Especially in the CTVA group, the $FeNO_{50}$ and $FeNO_{200}$ were significantly higher than the classic asthma ($P < .01$) and CVA groups ($P < .05$) (Figs. 2A–1, A-2).

Smoking exposure was found to increase the central and peripheral airway inflammation in asthma. The patients were divided into smoking, ex-smoking, nonsmoking subgroups in the classic and atypical patients with asthma. No differences were observed in terms of pattern of inflammation in the smoking, ex-smoking, and nonsmoking subgroups in classic asthma, CVA, and CTVA. However, the levels of $FeNO_{50}$ and $FeNO_{200}$ were highest in the smoking subgroups, followed by the ex-smoking subgroup and lowest in the nonsmoking subgroup (**Fig. 2B1-B3**). For FnNO and $CaNO_{dual}$, we did not find any such tendency pattern.

3.3. Exhaled NO associated with SAD in classic asthma, not in CVA and CTVA

Considering SAD could be affected by inflammation initially, we studied the relationship between the exhaled NO of FnNO, $FeNO_{50}$, $FeNO_{200}$, $CaNO_{dual}$, and SAD in the classic asthma, CVA and CTVA groups using Spearman's rank tests (**Table 2**). It was observed that $FeNO_{50}$ and $FeNO_{200}$ were associated with FEF_{25-75} % predicted, FEF_{50}, FEF_{50} % predicted, FEF_{75}, FEF_{75} % predicted, and RV/TLC in the classic asthma group. With an increase in the $FeNO_{50}$ and $FeNO_{200}$, the SAD-related variables such as FEF_{25-75} % predicted ($r = -0.292$, $r = -0.277$), FEF_{50} ($r = -0.199$, $r = -0.188$), FEF_{50} % predicted ($r = -0.294$, $r = -0.277$), FEF_{75} ($r = -0.173$, $r = -0.162$), FEF_{75} % predicted ($r = -0.263$, $r = -0.243$) decreased significantly and RV/TLC increased ($r = 0.169$, $r = -0.158$). However, no such correlations were observed between $FeNO_{50}$, $FeNO_{200}$ and SAD-related variables in the CVA and CTVA groups. The results suggested that CVA and CTVA might be the precursor of classic asthma. With the central and peripheral airway inflammation, early airway obstruction [35] and air trapping formed gradually in small airway in the development of asthma.

3.4. SAI is the risk for asthma development

We then assessed the risk factors in the development of asthma and inferred that the airway inflammation might be the main risk for asthma. The exhaled NO of FnNO, $FeNO_{50}$, $FeNO_{200}$, $CaNO_{dual}$, and PFT parameters such as FEV_1% predicted, FVC% predicted, FEV_1/FVC, and SAD-related variables such as FRC% predicted, RV% predicted, RV/TLC, RV/TLC% predicted, FEF_{25-75}, FEF_{25-75} % predicted, FEF_{50}, FEF_{50} % predicted, FEF_{75}, FEF_{75} % predicted, age, height, weight, and BMI were taken as covariates using logistic regression to determine the potential risk (**Table 3**, **detailed information in appendix**). $FeNO_{200}$ was the main risk (adjusted odds ratio [OR], 1.591; 95 % CI, 1.121–2.259; P = .009) in classic asthma and (adjusted OR, 1.456; 95 % CI, 1.247–1.700; P = .000) in CVA. Owing to the small number of CTVA group, no significant results were observed.

3.5. SAI is correlated to eosinophils in classic and atypical asthma

The potential mechanisms of SAI in classic and atypical asthma were studied. The correlation between exhaled NO of FnNO, $FeNO_{50}$, $FeNO_{200}$, $CaNO_{dual}$ and white cells counts was analyzed by using Spearman's rank tests to assess the inflammation type. The results showed that the percentage and absolute eosinophil count correlated with $FeNO_{50}$ ($P < .01$) and $FeNO_{200}$ ($P < .01$) in classic

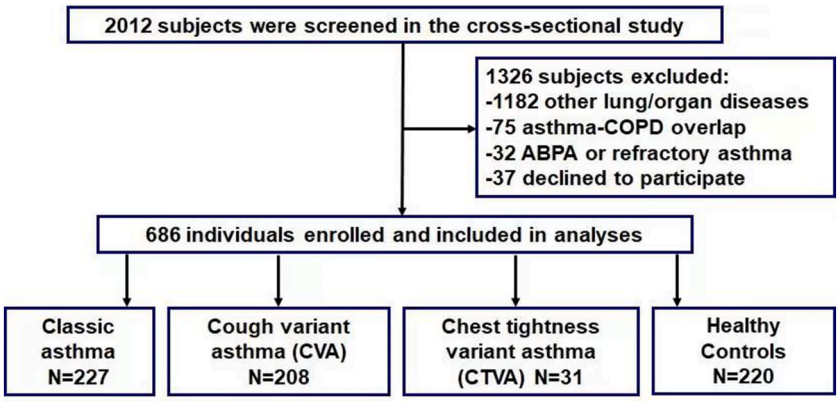

Fig. 1. Study profile. Number of people who were enrolled and analyzed in the study.

Table 1
Baseline characteristics.

Characteristics	Classic Asthma	CVA	CTVA	Controls
	N = 227	N = 208	N = 31	N = 220
Age (year, SD)	46.3 (15.6)	45.2 (15.6)	44.2 (13.6)	45.4 (14.8)
Gender, Male, n (%)	108 (47.6)	98 (47.1)	15 (47.8)	107 (48.6)
Female, n (%)	119 (52.4)	110 (52.9)	16 (52.2)	113 (51.4)
Height (cm, SD)	167.2 (9.2)	165.4 (8.8)	168.0 (8.5)	166.5 (7.9)
Weight (kg, SD)	67.3 (13.0)	65.4 (12.7)	65.3 (15.6)	65.8 (11.4)
BMI (SD)	24.0 (3.8)	23.8 (3.4)	22.9 (3.7)	23.3 (3.6)
Smoking status				
Ex-smoker, n (%)	27 (11.9)	10 (4.8)	6 (19.4)	–
Current smoker, n (%)	14 (6.2)	20 (9.6)	6 (19.4)	–
Never smoked, n (%)	186 (81.9)	178 (85.6)	19 (61.2)	220 (100)
Pulmonary Function Test				
FVC (SD), L	3.7 (1.0)	3.6 (1.0)	3.6 (0.8)	3.5 (0.9)
FVC (SD), % predicted	97.1 (14.5)	98.2 (13.9)	98.2 (12.9)	102.3 (12.4)
FEV_1 (SD), L	2.8 (0.8)	2.8 (0.8)	2.9 (0.8)	2.8 (0.8)
FEV_1 (SD), % predicted	90.8 (15.6)	95.1 (16.4)	95.8 (19.0)	102.1 (11.8)
FEV_1/FVC (SD), ratio	75.9 (8.0)	78.7 (8.5)	80.2 (11.6)	80.7 (9.9)
FEF_{25-75} (SD), % predicted	63.8 (24.3)	75.8 (28.0)	83.1 (27.1)	82.2 (22.2)
FEF_{50} (SD), % predicted	65.8 (23.2)	77.4 (28.2)	84.5 (24.1)	85.1 (21.7)
FEF_{75} (SD), % predicted	59.5 (28.8)	72.4 (35.0)	78.0 (36.1)	78.3 (27.9)
FRC, % predicted	109.1 (19.2)	107.6 (24.4)	116.9 (23.1)	111.7 (23.1)
RV, % predicted	115.4 (18.9)	106.8 (20.3)	120.2 (25.1)	110.5 (23.0)
TLC, % predicted	99.1 (10.3)	96.5 (14.3)	102.9 (8.9)	98.0 (18.4)
RV/TLC, % predicted	120.7 (19.8)	112.1 (19.1)	119.9 (23.2)	112.6 (18.7)

Data shown were means (SD) or as percentages. CVA, cough variant asthma; CTVA, chest tightness variant asthma; BMI, body mass index; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; FEF, forced expiratory flow; FRC, functional residual capacity; RV, residual volume; SD, standard deviation; TLC, total lung capacity.

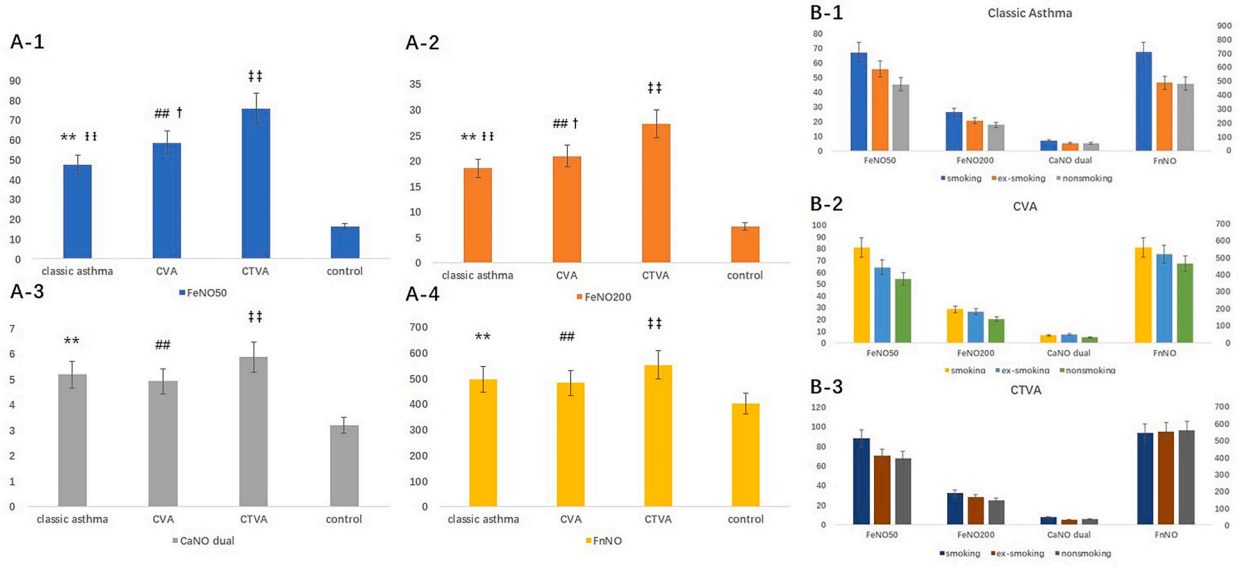

Fig. 2. Exhaled NO in asthma. A: The tendency of exhaled NO in classic asthma and atypical asthma. A-1: $FeNO_{50}$, A-2: $FeNO_{200}$, A-3: $CaNO_{dual}$, A-4: FnNO; B: The tendency of exhaled NO in smoking, ex-smoking and nonsmoking. ** $p<0.01$ classic asthma vs. control; ‡‡ $p<0.01$ classic asthma vs. CTVA; ## $p<0.01$ CVA vs. control; † $p<0.05$, CVA vs.CTVA; ‡ ‡ $p<0.01$ CTVA vs. control.

asthma as well as in CVA and CTVA (Table 4). The percentage of lymphocyte was significantly correlated with $FeNO_{50}$ and $FeNO_{200}$ ($P < .05$), but the absolute lymphocyte was not consistent in the CVA group. There was no correlation observed between the exhaled NO and IgE in the classic and atypical asthma groups.

Table 2
The correlation between exhaled NO and small airways dysfunction.

	$FeNO_{50}$		$FeNO_{200}$		$CaNO_{dual}$		FnNO	
	r	*p* value	r	*p* value	r	*p* value	r	*p* value
Classic asthma								
FEF_{25-75}, % predicted	−0.292	0.000	−0.277	0.000	−0.084	0.093	−0.105	0.063
FEF_{50}, L	−0.199	0.000	−0.188	0.000	−0.041	0.411	−0.023	0.648
FEF_{50}, % predicted	−0.294	0.000	−0.277	0.000	−0.079	0.114	−0.124	0.061
FEF_{75}, L	−0.173	0.000	−0.162	0.001	−0.026	0.601	−0.011	0.834
FEF_{75}, % predicted	−0.263	0.000	−0.243	0.000	−0.063	0.209	−0.141	0.054
FRC, % predicted	−0.074	0.141	−0.083	0.097	−0.134	0.074	−0.029	0.563
RV, % predicted	0.101	0.092	0.090	0.072	−0.014	0.785	0.030	0.557
TLC, % predicted	−0.008	0.869	−0.002	0.964	0.011	0.833	0.040	0.430
RV/TLC, % predicted	0.169	0.001	0.158	0.002	0.042	0.399	0.094	0.060
CVA								
FEF_{25-75}, % predicted	−0.072	0.159	−0.096	0.060	−0.072	0.156	−0.119	0.120
FEF_{50}, L	−0.010	0.845	−0.034	0.501	−0.017	0.737	−0.047	0.360
FEF_{50}, % predicted	−0.074	0.143	−0.111	0.095	−0.083	0.102	−0.114	0.062
FEF_{75}, L	−0.005	0.916	−0.012	0.812	−0.050	0.328	−0.052	0.313
FEF_{75}, % predicted	−0.066	0.195	−0.077	0.129	−0.067	0.185	−0.160	0.054
FRC, % predicted	−0.171	0.054	−0.210	0.062	−0.183	0.137	−0.046	0.388
RV, % predicted	−0.125	0.064	−0.146	0.052	−0.105	0.130	−0.048	0.367
TLC, % predicted	−0.052	0.321	−0.109	0.073	−0.167	0.120	0.052	0.321
RV/TLC, % predicted	−0.063	0.220	−0.063	0.221	−0.065	0.200	−0.059	0.249
CTVA								
FEF_{25-75}, % predicted	0.043	0.517	0.058	0.390	0.076	0.225	−0.009	0.893
FEF_{50}, L	0.068	0.307	0.092	0.168	0.123	0.065	0.055	0.416
FEF_{50}, % predicted	0.020	0.769	0.036	0.596	0.066	0.327	0.001	0.986
FEF_{75}, L	0.119	0.074	0.143	0.067	0.153	0.068	0.064	0.341
FEF_{75}, % predicted	0.039	0.564	0.051	0.449	0.087	0.193	−0.075	0.266
FRC, % predicted	0.003	0.966	0.021	0.759	−0.034	0.610	0.081	0.232
RV, % predicted	0.125	0.061	0.160	0.061	0.106	0.112	0.084	0.213
TLC, % predicted	0.030	0.654	0.069	0.301	0.147	0.082	0.119	0.078
RV/TLC, % predicted	0.183	0.060	0.209	0.081	0.102	0.129	0.052	0.444

CaNO: concentration of alveolar NO; CVA, cough variant asthma; CTVA, chest tightness variant asthma; FEF, forced expiratory flow; FeNO: fractional exhaled nitric oxide; FRC, functional residual capacity; NO, nitric oxide; RV, residual volume; TLC, total lung capacity.

Table 3
The risk for asthma development by logistic regression.

	Odds Ratio	95%CI	*p* value
Classic asthma			
FnNO	1.000	0.998–1.002	0.751
$FeNO_{50}$	0.972	0.873–1.082	0.605
$FeNO_{200}$	1.591	1.121–2.259	0.009
$CaNO_{dual}$	0.859	0.653–1.129	0.275
CVA			
FnNO	0.999	0.997–1.001	0.221
$FeNO_{50}$	1.004	0.972–1.038	0.807
$FeNO_{200}$	1.456	1.247–1.700	0.000
$CaNO_{dual}$	1.002	0.855–1.175	0.979

CVA, cough variant asthma; CTVA, chest tightness variant asthma; CaNO: concentration of alveolar NO; FeNO: fractional exhaled nitric oxide; NO: nitric oxide.

3.6. $FeNO_{200}$ is a better predictor of inflammation than $CaNO_{dual}$ for asthma

$FeNO_{200}$ and $CaNO_{dual}$ were measured for the peripheral airway/alveolar inflammation in the study. According to the ROC curve analysis in this study, $FeNO_{200}$ was a better predictor of peripheral airway/alveolar inflammation (ROC, AUC = 0.847) than $CaNO_{dual}$ (ROC AUC = 0.642) in the classic asthma group (Appendix, Figure S1 A). The sensitivity and specificity were 73.6 % and 81.9 % in $FeNO_{200}$ and 63.4 % and 59.7 % in $CaNO_{dual}$, respectively. In the CVA group also, $FeNO_{200}$ demonstrated better performance (ROC, AUC = 0.891) than $CaNO_{dual}$ (ROC AUC = 0.615) (Appendix, Figure S1 B). The sensitivity and specificity were 80.6 % and 81.9 % in $FeNO_{200}$ and 46.6 % and 73.1 % in $CaNO_{dual}$, respectively. Similar results were seen in the CTVA group. The AUC of $FeNO_{200}$ ROC was 0.994, whereas AUC of $CaNO_{dual}$ ROC was 0.619 (Appendix, Figure S1 C). The sensitivity and specificity were 93.5 % and 98.1 % in $FeNO_{200}$ and 41.9 % and 93.5 % in $CaNO_{dual}$, respectively.

Table 4
The correlation between exhaled NO and white cell classification.

	$FeNO_{50}$		$FeNO_{200}$		$CaNO_{dual}$		FnNO	
	r	*p* value	r	*p* value	r	*p* value	r	*p* value
Classic asthma								
White cell counts (*10^9/L)	0.052	0.399	0.078	0.208	0.120	0.053	0.004	0.953
Neutrophils (%)	−0.170	0.091	−0.141	0.129	0.033	0.599	0.041	0.507
Absolute Neutrophils (*10^9/L)	−0.020	0.745	0.005	0.931	0.080	0.199	0.019	0.756
Lymphocyte (%)	0.103	0.096	0.074	0.334	−0.075	0.226	−0.074	0.234
Absolute Lymphocyte (*10^9/L)	0.132	0.232	0.134	0.230	0.074	0.233	−0.059	0.341
Monocyte (%)	−0.035	0.574	−0.034	0.585	0.022	0.719	0.036	0.564
Absolute Monocyte (*10^9/L)	−0.045	0.467	−0.022	0.723	0.091	0.145	−0.114	0.068
Eosinophils (%)	**0.331**	**0.000**	**0.320**	**0.000**	0.116	0.061	0.070	0.258
Absolute Eosinophils (*10^9/L)	**0.274**	**0.000**	**0.269**	**0.001**	0.113	0.069	0.013	0.833
Basophils (%)	0.121	0.051	0.086	0.165	−0.070	0.259	−0.082	0.188
Absolute Basophils (*10^9/L)	0.098	0.114	0.079	0.205	−0.012	0.851	−0.098	0.114
IgE (kIU/L)	0.033	0.743	0.062	0.536	0.004	0.971	−0.035	0.726
CVA								
White cell counts (*10^9/L)	0.159	0.071	0.161	0.051	0.007	0.922	0.016	0.812
Neutrophils (%)	0.023	0.727	0.016	0.807	0.060	0.366	−0.044	0.510
Absolute Neutrophils (*10^9/L)	0.128	0.054	0.127	0.057	0.013	0.846	0.028	0.785
Lymphocyte (%)	−0.175	**0.008**	−0.168	**0.011**	−0.045	0.496	−0.013	0.844
Absolute Lymphocyte (*10^9/L)	−0.029	0.668	−0.016	0.806	0.015	0.823	−0.005	0.937
Monocyte (%)	−0.005	0.935	−0.001	0.983	0.025	0.704	0.101	0.131
Absolute Monocyte (*10^9/L)	0.052	0.441	0.062	0.355	0.039	0.564	−0.031	0.647
Eosinophils (%)	**0.501**	**0.000**	**0.499**	**0.000**	−0.058	0.382	0.127	0.058
Absolute Eosinophils (*10^9/L)	**0.471**	**0.000**	**0.473**	**0.000**	−0.042	0.535	0.076	0.261
Basophils (%)	0.101	0.131	0.107	0.107	−0.156	0.091	0.080	0.236
Absolute Basophils (*10^9/L)	0.111	0.123	0.111	0.123	−0.056	0.442	0.049	0.503
IgE (kIU/L)	0.203	0.064	0.162	0.142	−0.013	0.906	0.043	0.696
CTVA								
White cell counts (*10^9/L)	−0.010	0.920	−0.010	0.920	0.007	0.943	0.000	0.996
Neutrophils (%)	−0.085	0.383	−0.071	0.468	0.034	0.729	−0.069	0.480
Absolute Neutrophils (*10^9/L)	−0.066	0.495	−0.065	0.502	−0.028	0.776	−0.052	0.596
Lymphocyte (%)	−0.022	0.818	−0.039	0.687	−0.119	0.225	−0.010	0.921
Absolute Lymphocyte (*10^9/L)	0.016	0.873	0.007	0.943	−0.023	0.809	0.034	0.727
Monocyte (%)	0.070	0.470	0.088	0.366	0.099	0.310	0.047	0.626
Absolute Monocyte (*10^9/L)	−0.009	0.931	0.016	0.872	0.082	0.401	−0.143	0.145
Eosinophils (%)	**0.481**	**0.000**	**0.482**	**0.000**	**0.340**	**0.000**	**0.365**	**0.000**
Absolute Eosinophils (*10^9/L)	**0.387**	**0.000**	**0.398**	**0.000**	**0.322**	**0.001**	**0.256**	**0.008**
Basophils (%)	0.012	0.901	−0.030	0.762	−0.181	0.061	−0.050	0.608
Absolute Basophils (*10^9/L)	−0.008	0.934	−0.032	0.748	−0.096	0.328	−0.077	0.434
IgE (kIU/L)	0.027	0.862	0.122	0.429	0.194	0.206	0.165	0.285

CVA, cough variant asthma; CTVA, chest tightness variant asthma; CaNO: concentration of alveolar NO; FeNO: fractional exhaled nitric oxide; IgE, immunoglobulin E; NO: nitric oxide.

4. Discussion

With the exhaled NOs measurement, this cross-sectional study showed that the central and peripheral airway inflammation in CVA and CTVA was higher than classic asthma (Fig. 2A1-2). However, $FeNO_{50}$ and $FeNO_{200}$ were associated with SAD in classic asthma but not in CVA and CTVA (Table 2). Considering that SAD is initially caused by inflammation, CVA and CTVA might occur earlier than classic asthma and could eventually evolve into classic asthma if not treated. Furthermore, we found that SAI was the main risk in the development of asthma, which can be correlated to eosinophils. The smoking exposure increased the exhaled NO of $FeNO_{50}$ and $FeNO_{200}$ in classic asthma, CVA, and CTVA, whereas the smoking cessation decreased the central and peripheral airway inflammation. Finally, we also observed that $FeNO_{200}$, a biomarker of peripheral airway inflammation, was a better predictor of asthma than $CaNO_{dual}$ (Appendix, Figure S1 ABC).

The whole airway inflammations were increased in the classic and atypical asthma groups than control groups in our study (Fig. 2**A**). Interestingly, more severe inflammatory process was present in the central and peripheral airways in the CVA and CTVA groups compared with classic asthma group (Fig. 2A1-2). The potential pathophysiological mechanisms of CVA and CTVA were different from the classic asthma. The high level of central and peripheral airway inflammation, rather than the degree of airway hypersensitivity [36] and impairment of small airway function, was an important risk factor in the development of asthma in patients with CVA and CTVA.

FeNO levels are associated with the presence of inflammation in the lungs. We found that the exhaled NOs of $FeNO_{50}$ and $FeNO_{200}$ were associated with SAD in classic asthma but not in CVA and CTVA (Table 2). With an increase in the $FeNO_{50}$ and $FeNO_{200}$, the SAD-related variables such as FEF_{25-75} % predicted, FEF_{50}, FEF_{50} % predicted, FEF_{75}, FEF_{75} % predicted decreased and RV/TLC increased in classic asthma. Although SAD have now been recognized as a predominant site of airflow obstruction in many asthmatic diseases, we

did not find the exhaled NOs related to SAD in CVA and CTVA. Indeed, the number of CVA patients showed less small airway dysfunction than those of classic asthma [37]. Since inflammation plays a key role in the initiation of SAD, it was first confirmed using noninvasive methods that CVA and CTVA might be a pre-asthmatic condition. As consistent with a previous observational study, which that reported that patients with CVA eventually develop typical asthma in a span of 3–5 years [10].

The role of SAI and SAD in the development of asthma was also evaluated in the study. We found that SAI was the main risk for asthma. Further, the exhaled NO measured as FnNO, $FeNO_{50}$, $FeNO_{200}$ and $CaNO_{dual}$, PFT parameters, SAD-related variables, and age, height, weight, BMI were studied as covariates to determine the potential risks (**Table 3**). The results showed that $FeNO_{200}$ was the main risk in classic asthma (adjusted OR, 1.591; 95 % CI, 1.121–2.259; P = .009) and in CVA (adjusted OR, 1.456; 95 % CI, 1.247–1.700; P = .000). Owing to the fractional concentration of exhaled NO was one of the accurate methods to assess the airway inflammation noninvasively [38], SAI might be a sensitive indicator of early disease rather than spirometry [23]. Although peripheral airway inflammation was reported in severe symptomatic, steroid-dependent asthma [38,39], attention should also be paid to the pre-asthmatic state (CVA and CTVA).

Indeed, patients with asthma who have disproportionate SAI and impairment of small airway function are difficult to treat. The narrowing of small airways is narrowing, often caused by smooth muscle contraction after inflammation exudation by inhalation of allergic and nonallergic irritants in asthmatics [40]. Furthermore, small airways remodeling could affect the airway wall stiffness, changing their distensibility. The extracellular matrix has been found throughout the lung in patients with fatal asthma. It is well known extra-fine particles <2 μm would result in increased peripheral deposition. As SAI was the main risk in the development of asthma, extra-fine particles may improve asthma by enhancing deposition in the small airways. In addition, SAI is correlated to eosinophils in classic and atypical asthma, the extra-fine inhaled ICS therapies or biologics would be selective for asthma treatment.

It was reported that the prevalence rates for cigarette smoking in asthmatics were similar to that of the general population [41], and in many developed countries more than 25% of adults with asthma were current smokers. Previous study showed that cigarette smoking was the independent risk factor in asthma (OR, 1.89; 95% CI, 1.26–2.84; $P = .004$). Asthma and active cigarette smoking interact to cause more severe symptoms and accelerate a decline in lung function [42]. Cigarette smoking may alter the inflammation that was associated with asthma, although there are limited published data on airway pathology in smokers compared with non-smokers with asthma. Our study confirmed the results that cigarette smoking resulted in increased exhaled NO of $FeNO_{50}$ and $FeNO_{200}$ in classic and atypical asthma, whereas smoking cessation decreased the central and peripheral airway inflammation; $FeNO_{50}$ and $FeNO_{200}$ were lowest in the nonsmoking groups (Fig. 2B1-3). Therefore, every effort should be made to encourage patients with asthma to stop smoking [15].

Eosinophils are considered to play a central pathogenetic role in asthma. A previous study reported that eosinophilic inflammation was correlated with the clinical severity of asthma [43]. High FeNO levels in combination with elevated eosinophil count and prior exacerbations were associated with a greater risk of severe asthma exacerbations [44]. Both biomarkers, blood eosinophils and the exhaled NO, provided different and complementary mechanistic information. Blood eosinophils reflected the systemic pool of available effector cells and circulating interleukins, whereas the exhaled NO reflects airway type 2 inflammatory activity and the chemotactic pull to the airways [45]. Eosinophilic inflammation was also observed in patients with CVA [46], CTVA [11] as well as in classic asthma. Our study also identified correlation between these two biomarkers. The percentage and absolute eosinophils were significantly correlated with exhaled NO of $FeNO_{50}$ ($P < .01$) and $FeNO_{200}$ ($P < .01$) not only in classic asthma, but also in CVA and CTVA (**Table 4**).

A previous study reported that CaNO was related to features of allergic inflammation in asthmatic diseases, whereas $FeNO_{200}$ was proportional to CaNO, mainly for the evaluation of peripheral airway/alveolar inflammation [26,27]. Our study showed that $FeNO_{200}$ was a better predictor (ROC, AUC = 0.847) than $CaNO_{dual}$ (ROC, AUC = 0.642) in the classic asthma group. $FeNO_{200}$ also performed well in the CVA and CTVA groups (Appendix, Figure S1 BC). These data suggested that $FeNO_{200,}$ as the direct measured value, would be a more accurate predictive tool for assessing peripheral airway inflammation compared with $CaNO_{dual}$ in classic and atypical asthma, but $CaNO_{dual}$ as an indicator of SAI still has some role in the clinical assessment.

As our study was limited by the small number of CTVA group, no significant results were observed in the statistical analysis such as CTVA in relation to potential risk and smoking status. While the central and peripheral airway inflammation in the CTVA group were significantly higher than the classic asthma (P < .01) and CVA groups (P < .05) which suggest that early diagnosis and timely treatment for CTVA are important. Some SAD variables including RV need to be evaluated indirectly through body plethysmography, which cannot be measured directly with a spirometer [47]. Future studies with larger cohort size would be needed to confirm the results. It remains to explore the mechanisms of eosinophilia and the underlying type 2 inflammation in CVA and CTVA.

5. Conclusions

Our study findings indicate that a more severe inflammatory process was present in the central and peripheral airways in patients with CVA and CTVA compared to classic asthma, as measured by FeNO levels. The levels of $FeNO_{50}$ and $FeNO_{200}$ were linked to SAD in classic asthma, while this association was not observed in CVA and CTVA. This suggests that CVA and CTVA could potentially represent pre-asthmatic conditions, and this was demonstrated using noninvasive methods. Additionally, our findings suggest that cigarette smoking may worsen airway inflammation in both classic and atypical asthma. It was also observed that SAI was the primary risk factor in the development of asthma preceding SAD, which may be related to eosinophils. In future studies like advanced micro-imaging techniques, biomarker discovery, air pollution and environment exposures, precision medicine are the mainly focusing on the SAI in asthma.

Fundings

This study was supported by the Construction of Multidisciplinary Cooperative Diagnosis and Treatment for Severe Pneumonia, Number: W2020-013; The Mechanisms of Antibiotic Resistance in Severe Pneumonia Treated with Traditional Chinese and Western Medicine, Number: ZY (2021-2023)-0207-01; Multi-dimensional Model in Standardized Training of Respiratory and Critical Medical Specialists, Number: FDYXYBJ-20222009; Science and Technology Commission of Shanghai Municipality, Number: 20DZ2261200; National key R&D plan, Number: 2020YFC2003700; Shanghai Municipal Key Clinical Specialty, Number: shslczdzk02201.

Availability of data and materials

All data or resources used in the current study are in Zhongshan Hospital, Fudan University (https://www.zs-hospital.sh.cn/), which has not been deposited into a publicly available repository. In addition, the data that support the findings of this study are available from the corresponding author Jun She upon reasonable request.

CRediT authorship contribution statement

Junjie Han: Data curation. **Li Li:** Data curation. **Ying Gong:** Formal analysis. **Juan Song:** Formal analysis. **Yichun Zhu:** Formal analysis. **Cuicui Chen:** Formal analysis. **Lin Shi:** Formal analysis. **Jian Wang:** Formal analysis. **Yuanlin Song:** Writing – review & editing, Conceptualization. **Jun She:** Writing – original draft, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Li Ji and Jin Linjie for their technical support of the exhaled NO measurement.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36124.

References

[1] Global Initiative for Asthma, "2022 GINA MAIN REPORT," Global Initiative for Asthma - GINA (n.d.). https://ginasthma.org/gina-reports/(accessed May 31, 2023).
[2] Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015, Lancet Respir. Med. 5 (2017) 691–706, https://doi.org/10.1016/S2213-2600(17)30293-X.
[3] T. Mauad, E.H. Bel, P.J. Sterk, Asthma therapy and airway remodeling, J. Allergy Clin. Immunol. 120 (2007) 997–1009, https://doi.org/10.1016/j.jaci.2007.06.031.
[4] M. Kraft, The distal airways: are they important in asthma? Eur. Respir. J. 14 (1999) 1403–1417, https://doi.org/10.1183/09031936.99.14614039.
[5] R.J. Martin, Therapeutic significance of distal airway inflammation in asthma, J. Allergy Clin. Immunol. 109 (2002) S447–S460, https://doi.org/10.1067/mai.2002.121409.
[6] M. van den Berge, N.H.T. Ten Hacken, J. Cohen, W.R. Douma, D.S. Postma, Small airway disease in asthma and COPD: clinical implications, Chest 139 (2011) 412–423, https://doi.org/10.1378/chest.10-1210.
[7] N. Scichilone, S. Battaglia, D. Olivieri, V. Bellia, The role of small airways in monitoring the response to asthma treatment: what is beyond FEV1? Allergy 64 (2009) 1563–1569, https://doi.org/10.1111/j.1398-9995.2009.02139.x.
[8] E. van der Wiel, N.H.T. ten Hacken, D.S. Postma, M. van den Berge, Small-airways dysfunction associates with respiratory symptoms and clinical features of asthma: a systematic review, J. Allergy Clin. Immunol. 131 (2013) 646–657, https://doi.org/10.1016/j.jaci.2012.12.1567.
[9] B. Lipworth, A. Manoharan, W. Anderson, Unlocking the quiet zone: the small airway asthma phenotype, Lancet Respir. Med. 2 (2014) 497–506, https://doi.org/10.1016/S2213-2600(14)70103-1.
[10] M. Fujimura, H. Ogawa, Y. Nishizawa, K. Nishi, Comparison of atopic cough with cough variant asthma: is atopic cough a precursor of asthma? Thorax 58 (2003) 14–18, https://doi.org/10.1136/thorax.58.1.14.
[11] F. Yan, W. Li, W.-J. Guan, M. Chen, C. Qiu, W. Tang, X. Liu, X. Xiang, J. Li, M. Jin, Y. Dai, P. Chen, X. Wu, Z. Qiu, L. Dong, L. Zhao, X. Lin, C. Wu, B. Wu, Y. Yuan, F. Shi, T. Zhang, J. Zhou, M. Xie, X. Fang, H. Zhang, B. Xiao, M. Xian, J. Wang, Z. Qiu, J. Lin, B. Ji, Y. Zhou, Y. Li, C. Liu, Y. Chen, Y. Zeng, L. Liu, W. Hua, H. Huang, J. Zhou, Y. Hu, L. Che, S. Ying, Z. Chen, N. Zhong, H. Shen, Response of patients with chest tightness variant asthma with routine asthma treatment regimen: a 1-year multicenter, prospective, real-world study, Clin. Transl. Med. 10 (2020) e178, https://doi.org/10.1002/ctm2.178.
[12] H. Taniguchi, H. Furuse, Y. Nakanishi, T. Tsuda, K. Totsuka, Y. Masaki, K. Suzuki, S. Ishizawa, H. Miyazawa, Bronchial biopsy and reactivity in patients with chest tightness relieved with bronchodilator, J. Asthma 54 (2017) 479–487, https://doi.org/10.1080/02770903.2016.1236940.
[13] M.D. Lougheed, S.E. Turcotte, T. Fisher, Cough variant asthma: lessons learned from deep inspirations, Lung 190 (2012) 17–22, https://doi.org/10.1007/s00408-011-9348-6.
[14] R. Chaudhuri, E. Livingston, A.D. McMahon, J. Lafferty, I. Fraser, M. Spears, C.P. McSharry, N.C. Thomson, Effects of smoking cessation on lung function and airway inflammation in smokers with asthma, Am. J. Respir. Crit. Care Med. 174 (2006) 127–133, https://doi.org/10.1164/rccm.200510-1589OC.

[15] N.C. Thomson, R. Chaudhuri, E. Livingston, Asthma and cigarette smoking, Eur. Respir. J. 24 (2004) 822–833, https://doi.org/10.1183/09031936.04.00039004.

[16] M.A. Bhat, Indoor microplastics: a comprehensive review and bibliometric analysis, Environ. Sci. Pollut. Res. 30 (2023) 121269–121291, https://doi.org/10.1007/s11356-023-30902-0.

[17] M.A. Bhat, A comprehensive characterization of indoor ambient microplastics in households during the COVID-19 pandemic, Air Qual Atmos Health (2024), https://doi.org/10.1007/s11869-024-01559-6.

[18] D. Ekmekcioglu, S.S. Keskin, Characterization of indoor air particulate matter in selected elementary schools in Istanbul, Turkey, Indoor Built Environ. 16 (2007) 169–176, https://doi.org/10.1177/1420326X07076777.

[19] M.A. Bhat, Airborne microplastic contamination across diverse university indoor environments: a comprehensive ambient analysis, Air Qual Atmos Health (2024), https://doi.org/10.1007/s11869-024-01548-9.

[20] M.A. Bhat, Microplastics in indoor deposition samples in university classrooms, Discov Environ 2 (2024) 23, https://doi.org/10.1007/s44274-024-00054-0.

[21] M.A. Bhat, Unravelling the microplastic contamination: a comprehensive analysis of microplastics in indoor house dust, Indoor Built Environ. (2024) 1420326X241248054, https://doi.org/10.1177/1420326X241248054.

[22] M.A. Bhat, Indoor microplastics and microfibers sources and impacts on human health, in: Microfibre Pollution from Textiles, first ed., CRC Press, Boca Raton, 2024, pp. 285–307, https://doi.org/10.1201/9781003331995-16.

[23] W.J. Anderson, E. Zajda, B.J. Lipworth, Are we overlooking persistent small airways dysfunction in community-managed asthma? Ann. Allergy Asthma Immunol. 109 (2012) 185–189.e2, https://doi.org/10.1016/j.anai.2012.06.022.

[24] H. Rupani, B.D. Kent, Using fractional exhaled nitric oxide measurement in clinical asthma management, Chest 161 (2022) 906–917, https://doi.org/10.1016/j.chest.2021.10.015.

[25] I. Horváth, P.J. Barnes, S. Loukides, P.J. Sterk, M. Högman, A.-C. Olin, A. Amann, B. Antus, E. Baraldi, A. Bikov, A.W. Boots, L.D. Bos, P. Brinkman, C. Bucca, G. E. Carpagnano, M. Corradi, S. Cristescu, J.C. de Jongste, A.-T. Dinh-Xuan, E. Dompeling, N. Fens, S. Fowler, J.M. Hohlfeld, O. Holz, Q. Jöbsis, K. Van De Kant, H. H. Knobel, K. Kostikas, L. Lehtimäki, J. Lundberg, P. Montuschi, A. Van Muylem, G. Pennazza, P. Reinhold, F.L.M. Ricciardolo, P. Rosias, M. Santonico, M.P. van der Schee, F.-J. van Schooten, A. Spanevello, T. Tonia, T.J. Vink, A European Respiratory Society technical standard: exhaled biomarkers in lung disease, Eur. Respir. J. 49 (2017) 1600965, https://doi.org/10.1183/13993003.00965-2016.

[26] C. Delclaux, B. Mahut, F. Zerah-Lancner, C. Delacourt, S. Laoud, D. Cherqui, C. Duvoux, A. Mallat, A. Harf, Increased nitric oxide output from alveolar origin during liver cirrhosis versus bronchial source during asthma, Am. J. Respir. Crit. Care Med. 165 (2002) 332–337, https://doi.org/10.1164/ajrccm.165.3.2107017.

[27] X. Fan, N. Zhao, Z. Yu, H. Yu, B. Yin, L. Zou, Y. Zhao, X. Qian, X. Sai, C. Qin, C. Fu, C. Hu, T. Di, Y. Yang, Y. Wu, T. Bian, Clinical utility of central and peripheral airway nitric oxide in aging patients with stable and acute exacerbated chronic obstructive pulmonary disease, Int. J. Gen. Med. 14 (2021) 571–580, https://doi.org/10.2147/IJGM.S284688.

[28] C. Cao, W. Li, W. Hua, F. Yan, H. Zhang, H. Huang, Y. Ying, N. Li, F. Lan, S. Wang, X. Chen, J. Li, J. Liu, T. Lai, Z. Bao, Y. Cao, Y. Zhao, G. Huang, L. Huang, Y. Huang, P. Wu, C. Peng, Z. Chen, K.F. Chung, N. Zhong, S. Ying, H. Shen, Proteomic analysis of sputum reveals novel biomarkers for various presentations of asthma, J. Transl. Med. 15 (2017) 171, https://doi.org/10.1186/s12967-017-1264-y.

[29] Global Initiative for Chronic Obstructive Lung Disease "2022 GOLD REPORTS," Global Initiative for Chronic Obstructive Lung Disease - GOLD (n.d.). https://goldcopd.org/2022-gold-reports-2/(accessed May 31, 2023).

[30] P. Paredi, S.A. Kharitonov, S. Meah, P.J. Barnes, O.S. Usmani, A novel approach to partition central and peripheral airway nitric oxide, Chest 145 (2014) 113–119, https://doi.org/10.1378/chest.13-0843.

[31] American Thoracic Society, European Respiratory Society, ATS/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. 171 (2005) 912–930, https://doi.org/10.1164/rccm.200406-710ST.

[32] Chronic Airway Disease Committee of China Medical Education Association and China Asthma Alliance, Chinese expert consensus on the detection of exhaled nitric oxide and its application in the diagnosis and treatment of airway diseases 2021, Chin. Med. J. 101 (2021) 3092–3114.

[33] D.S. Postma, C. Brightling, S. Baldi, M. Van den Berge, L.M. Fabbri, A. Gagnatelli, A. Papi, T. Van der Molen, K.F. Rabe, S. Siddiqui, D. Singh, G. Nicolini, M. Kraft, ATLANTIS study group, Exploring the relevance and extent of small airways dysfunction in asthma (ATLANTIS): baseline data from a prospective cohort study, Lancet Respir. Med. 7 (2019) 402–416, https://doi.org/10.1016/S2213-2600(19)30049-9.

[34] J. She, P. Yang, Y. Wang, X. Qin, J. Fan, Y. Wang, G. Gao, G. Luo, K. Ma, B. Li, C. Li, X. Wang, Y. Song, C. Bai, Chinese water-pipe smoking and the risk of COPD, Chest 146 (2014) 924–931, https://doi.org/10.1378/chest.13-1499.

[35] M. Malerba, A. Radaeli, A. Olivini, G. Damiani, B. Ragnoli, V. Sorbello, F.L.M. Ricciardolo, Association of FEF25-75% impairment with bronchial hyperresponsiveness and airway inflammation in subjects with asthma-like symptoms, Respiration 91 (2016) 206–214, https://doi.org/10.1159/000443797.

[36] Y.Y. Koh, Y. Park, C.K. Kim, The importance of maximal airway response to methacholine in the prediction of wheezing development in patients with cough-variant asthma, Allergy 57 (2002) 1165–1170, https://doi.org/10.1034/j.1398-9995.2002.23602.x.

[37] J. Gao, H. Wu, F. Wu, Small airway dysfunction in patients with cough variant asthma: a retrospective cohort study, BMC Pulm. Med. 21 (2021) 49, https://doi.org/10.1186/s12890-021-01419-4.

[38] R.A. Dweik, P.B. Boggs, S.C. Erzurum, C.G. Irvin, M.W. Leigh, J.O. Lundberg, A.-C. Olin, A.L. Plummer, D.R. Taylor, American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications, an official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications, Am. J. Respir. Crit. Care Med. 184 (2011) 602–615, https://doi.org/10.1164/rccm.9120-11ST.

[39] B.J. Lipworth, P.M. Short, P.A. Williamson, K.L. Clearie, T.C. Fardon, C.M. Jackson, A randomized primary care trial of steroid titration against mannitol in persistent asthma: STAMINA trial, Chest 141 (2012) 607–615, https://doi.org/10.1378/chest.11-1748.

[40] S.D. Anderson, K. Holzer, Exercise-induced asthma: is it the right diagnosis in elite athletes? J. Allergy Clin. Immunol. 106 (2000) 419–428, https://doi.org/10.1067/mai.2000.108914.

[41] C. Lemiere, L.-P. Boulet, Cigarette smoking and asthma: a dangerous mix, Cancer Res. J. 12 (2005) 79–80, https://doi.org/10.1155/2005/179032.

[42] K. Huang, T. Yang, J. Xu, L. Yang, J. Zhao, X. Zhang, C. Bai, J. Kang, P. Ran, H. Shen, F. Wen, Y. Chen, T. Sun, G. Shan, Y. Lin, G. Xu, S. Wu, C. Wang, R. Wang, Z. Shi, Y. Xu, X. Ye, Y. Song, Q. Wang, Y. Zhou, W. Li, L. Ding, C. Wan, W. Yao, Y. Guo, F. Xiao, Y. Lu, X. Peng, B. Zhang, D. Xiao, Z. Wang, Z. Chen, X. Bu, H. Zhang, X. Zhang, L. An, S. Zhang, J. Zhu, Z. Cao, Q. Zhan, Y. Yang, L. Liang, X. Tong, H. Dai, B. Cao, T. Wu, K.F. Chung, J. He, C. Wang, China Pulmonary Health (CPH) Study Group, Prevalence, risk factors, and management of asthma in China: a national cross-sectional study, Lancet 394 (2019) 407–418, https://doi.org/10.1016/S0140-6736(19)31147-X.

[43] J. Bousquet, P. Chanez, J.Y. Lacoste, G. Barnéon, N. Ghavanian, I. Enander, P. Venge, S. Ahlstedt, J. Simony-Lafontaine, P. Godard, Eosinophilic inflammation in asthma, N. Engl. J. Med. 323 (1990) 1033–1039, https://doi.org/10.1056/NEJM199010113231505.

[44] W.W. Busse, S.E. Wenzel, T.B. Casale, J.M. FitzGerald, M.S. Rice, N. Daizadeh, Y. Deniz, N. Patel, S. Harel, P.J. Rowe, N.M.H. Graham, T. O'Riordan, I.D. Pavord, Baseline FeNO as a prognostic biomarker for subsequent severe asthma exacerbations in patients with uncontrolled, moderate-to-severe asthma receiving placebo in the LIBERTY ASTHMA QUEST study: a post-hoc analysis, Lancet Respir. Med. 9 (2021) 1165–1173, https://doi.org/10.1016/S2213-2600(21)00124-7.

[45] S. Couillard, A. Laugerud, M. Jabeen, S. Ramakrishnan, J. Melhorn, T. Hinks, I. Pavord, Derivation of a prototype asthma attack risk scale centred on blood eosinophils and exhaled nitric oxide, Thorax 77 (2022) 199–202, https://doi.org/10.1136/thoraxjnl-2021-217325.

[46] A. Niimi, R. Amitani, K. Suzuki, E. Tanaka, T. Murayama, F. Kuze, Eosinophilic inflammation in cough variant asthma, Eur. Respir. J. 11 (1998) 1064–1069, https://doi.org/10.1183/09031936.98.11051064.

[47] K. Konstantinos Katsoulis, K. Kostikas, T. Kontakiotis, Techniques for assessing small airways function: possible applications in asthma and COPD, Respir. Med. 119 (2016) e2–e9, https://doi.org/10.1016/j.rmed.2013.05.003.