Small Airway Dysfunction in Asthma Is Associated with Perceived Respiratory Symptoms, Non-Type 2 Airway Inflammation, and Poor Responses to Therapy

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Keywords
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Abstract
Background: Emerging evidence has indicated that small airway dysfunction (SAD) contributes to the clinical expression of asthma. Objectives: The aim of the study was to explore the relationships of SAD assessed by forced expiratory flow between 25 and 75% (FEF25–75%), with clinical and inflammatory profile and treatment responsiveness in asthma. Method: In study I, dyspnea intensity (Borg scale), chest tightness, wheezing and cough (visual analog scales, VASs), and pre- and post-methacholine challenge testing (MCT) were analyzed in asthma patients with SAD and non-SAD. In study II, asthma subjects with SAD and non-SAD underwent sputum induction, and inflammatory mediators in sputum were detected. Asthma patients with SAD and non-SAD receiving fixed treatments were prospectively followed up for 4 weeks in study III. Spirometry, Asthma Control Questionnaire (ACQ), and Asthma Control Test (ACT) were carried out to define treatment responsiveness. Results: SAD subjects had more elevated ΔVAS for dyspnea ($p = 0.027$) and chest tightness ($p = 0.032$) after MCT. Asthma patients with SAD had significantly elevated interferon (IFN)-γ in sputum ($p < 0.05$), and Spearman partial correlation found FEF25–75% significantly related to IFN-γ and interleukin-8 (both having $p < 0.05$). Furthermore, multivariable regression analysis indicated SAD was significantly associated with worse

treatment responses (decrease in ACQ ≥0.5 and increase in ACT ≥3) (p = 0.022 and p = 0.032). Conclusions: This study indicates that SAD in asthma predisposes patients to greater dyspnea intensity and chest tightness during bronchoconstriction. SAD patients with asthma are characterized by non-type 2 inflammation that may account for poor responsiveness to therapy.

Introduction

Asthma is the most common chronic respiratory disease associated with airway hyperresponsiveness that leads to recurrent episodes of dyspnea, wheezing, chest tightness, and cough, with variable and often reversible airflow limitation [1]. It is known that the small airways are involved in asthma for a long time [2–4]. However, the contribution of the small airways to total lung resistance was minimal, and the small airways were always neglected and labeled the ‘‘quiet zone” [5]. Currently, there has been renewed interest in the role of small airway disease in asthma as emerging evidence has become available indicating that abnormalities in the small airways contribute to the clinical expression of asthma [6, 7].

The lungs are a branching structure, segmentally divided from the trachea (airway generation 1) to the alveoli (generation 23); the small airways are defined to have an internal diameter less than 2 mm (comprising generations 8–23) [8]. Until now, there have been no gold standard tools available to assess small airway dysfunction (SAD) [9, 10]. Methods for small airway assessment include lung function tests, biomarker measurement, and imaging techniques [7–12]. The mean forced expiratory flow (FEF) between 25 and 75% of forced vital capacity
(FEF25–75) is one of the most popular indices of small airway impairment, which is commonly available and easy to apply in clinical practice and sensitive to small airway obstruction [9, 10, 12].

Asthma guidelines emphasize both symptom control and future risk of asthma outcomes [1]. Symptoms such as dyspnea, wheezing, chest tightness, and cough are the key components for assessing asthma control [1]. Some studies found that SAD is associated with airway hyperresponsiveness, asthma severity, poor asthma control, and elevated exacerbations [6, 7, 13]. It has also been found that small airway function might be associated with responses to therapy in asthma patients [14]. Furthermore, although the presence of SAD in asymptomatic asthmatics has been recognized for a long time, the small airway phenotype of asthma has recently been defined as poorly controlled asthma, preserved FEV1 higher than 80% predicted with evidence of an impaired small airway function [7]. Meanwhile, most of these were retrospective, cross-sectional, or case-control studies [15–19], and the direct relationship between SAD and asthma symptoms or treatment response has been largely unexplored [14, 20].

Whether airway inflammation is related to SAD has also not been clarified [9]. Although some studies indicated a relationship between eosinophilic inflammation and SAD [21–23], evidence directly relating peripheral airway disease with eosinophilic airway inflammation is still lacking. Moreover, Farah et al. [24] found that neutrophilic airway inflammation was associated with dysfunction of the peripheral, diffusion-dependent acinar airways in stable asthma. Thus, the function of peripheral airways in asthma is potentially affected by different airway inflammation profiles, but its association with airway inflammation is not conclusive. Accordingly, we carried out this study of a clinical population with stable asthma

in China, the purpose of which was to investigate the relationship of SAD with perceived respiratory symptoms, airway inflammation, and response to therapy.

Methods

Design Overview and Subjects

This study of a clinical population with stable asthma in China included 3 parts, which was conducted from July 2012 to January 2018. Study I was a cross-sectional study to explore the effects of SAD on perceived respiratory symptoms in stable asthma patients during bronchoconstriction who were continuously recruited from the Asthma Clinic of West China Hospital at Sichuan University. Asthma symptoms and lung function assessments were performed both pre- and post-methacholine challenge testing (MCT), and the impact of SAD on respiratory symptoms was evaluated. The goal of study II, a cross-sectional study, was to assess the relationship between SAD and airway inflammatory profiles as measured by cell counts and inflammatory cytokines in induced sputum in adults with asthma who underwent sputum induction from the Australasian Severe Asthma Network (ASAN). Study III, a prospective cohort design, aimed to explore the relationship of SAD with treatment responses in a subset of subjects following fixed treatments with inhaled corticosteroids (ICs)/long-acting beta-agonists (LABA) plus leukotriene receptor antagonist (LTRA) from study II. Asthma was diagnosed according to American Thoracic Society (ATS) [25] guidelines and Global Initiative for Asthma (GINA) [1]. Adult patients (≥18 years of age) with stable asthma and preserved FEV1 (FEV1 ≥80% predicted) were included. The inclusion and exclusion of 3 parts in this study were described in detail in the supplementary file. The Institutional Review Board at the West China Hospital approved all protocols (Nos. 2014-30 and 2013-65), and written informed consent was obtained from all subjects.

Assessment of SAD and Respiratory Symptoms

Spirometry and MCT (APS, Jaeger, Wurzburg, Germany) were performed according to ATS recommendations [26–28]. Subjects inhaled increasing doses of Mch, delivered via a Rosenthal dosimeter starting at 0.015 mg and with maximal dose of 0.9 mg in an iterative increment fashion. Therefore, the cumulative dose of Mch causing a 20% decrease in FEV1 (provocative dose of methacholine required to achieve a 20% fall in FEV1 [PD20] FEV1-Mch) ranged from 0.015 to 2.565 mg [26]. We terminated challenge testing at the first occurrence of the following: FEV1 decreased by ≥20% of the baseline value, the highest dosage of Mch was administered, or the patient asked to stop due to intolerable symptoms. To reverse bronchoconstriction, salbutamol (100–200 μg) (GSK, Avda de Extremadura, Spain) was administered by a metered-dose inhaler every 10–15 min until the FEV1 fell within 10% of the baseline value.

In this study, the FEF25–75 <65% of predicted (FEF25–75, % pred) was defined as the cutoff point for identifying of SAD [28–31]. The intensity of dyspnea was evaluated by the modified Borg scale with items ranging from 1 (no dyspnea) to 10 (the most severe dyspnea that the subjects previously experienced or could imagine) [32, 33]. Dyspnea, chest tightness, wheezing, and cough intensity were also evaluated using the visual analog scale (VAS) pre- and post-MCT [34, 35]. Individuals were asked to place a mark perpendicular to the line at a position corresponding to their subjective assessment ranging from 0 (no symptoms at all) to 100 (the worst symptoms imaginable).

Sputum Induction, Analysis, and Cytokine Detection

Sputum induction and processing were performed based on standard methods, as described in our previous study [36]. Selected sputum was dispersed using dithiothreitol, and the sputum supernatant was aspirated and stored immediately at −80°C for subsequent

detection. Total cell counts and differential cell counts were performed with a centrifugation smear (CYTOPRO 7620, WESCOR®, INC, LOGAN, USA), and stained samples were prepared by well-trained Chinese and Australian laboratory researchers. Interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-8, IL-13, IL-17, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ in sputum supernatants were detected by a Luminex-based MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic bead Panel Kit (EMD Millipore Corporation, Billerica, MA, USA) and analyzed using Milliplex Analyst 5.1 software. It has been demonstrated that the Luminex-based xMAP® panel can be used for multianalyte profiling of sputum using the routinely applied method of sputum processing with dithiothreitol DTT [37, 38].

Assessment of Treatment Responsiveness

To assess responsiveness to therapy across patients with SAD and non-SAD in study III, subjects receiving one week of washout period followed by fixed treatments with ICs/LABA plus LTRA were prospectively followed up for 4 weeks. Subjects were excluded from the analysis if they changed their treatment within this period. Spirometry, Asthma Control Questionnaire (ACQ), and Asthma Control Test (ACT) were carried out to define treatment responsiveness as one or more of the following: ≥0.5 point decrease in ACQ, ≥3.0 point increase in ACT, or ≥12% increase of FEV1 [39]. The assessors were blinded to the results of SAD or non-SAD testing.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation or medians (interquartile ranges), depending on their distribution, and differences of respiratory symptoms, airway inflammation, and treatment response between groups were analyzed using either unpaired Student's t-tests or nonparametric statistics for continuous variables that were not normally distributed. Categorical data were compared using the $\chi 2$ test or Fisher's test. Spearman's partial correlation was used to describe the specific correlations between airway inflammation profiles and FEF25–75 (L/s or % predicted). Multiple logistic regression models were applied to explore the influence of SAD on the changes in respiratory symptoms during MCT and treatment response.

Theoretically, the lower limits of normal (LLNs) is the reference standard to define SAD in terms of FEF25–75 [40, 41], so we performed sensitivity analyses of primary outcomes in subjects with SAD and non-SAD grouped by FEF25–75% < LLN or not across all parts of this study. All figures were produced by GraphPad Prism software version 6 (GraphPad, USA). Statistical analysis was performed using Stata 13.0 software for Mac (Stata Corporation, College Station, TX, USA) and SPSS statistics 20.0 software (IBM, USA). Hypotheses were tested against two-sided alternatives, with $p \leq 0.05$ considered to be statistically significant.

Results

Clinical Characteristics of Asthma Patients with SAD

A total of 185 subjects were included in study I. According to our prespecified definition of SAD, subjects were divided into the non-SAD group (n = 62) and the SAD group (n = 123). The subjects with SAD were older, had greater BMI, longer asthma duration, poorer asthma control and Asthma Quality of Life Questionnaire (AQLQ), and higher asthma treatment step scores in GINA (all $p < 0.05$) (Table 1). However, there were no differences in PD20, VAS scores for cough, or asthma exacerbations within the previous 12 months across groups.

Impacts of SAD on Symptom Perception

The changes in FVC (L) and FEV1 (%) from pre- to post-MCT were similar across both groups. ΔFEV1 (L) ($p < 0.001$), ΔFEV1/FVC (%) ($p < 0.001$), and ΔFEF25–75 (%) ($p < 0.001$) in individuals with small airway impairment had a smaller decrease in physiologic response to

Table 1. Demographic, clinical, and physical characteristics in patients with asthma grouped by SAD in study I

Variable	Non-SAD	SAD	p value
N	62	123	
Age, yrs	31.6±12.2	40.6±11.1	<0.001
Sex, M/F BMI, kg/m2	32/30		
21.70±3.02	41/82		
23.93±3.47	0.016		
<0.001			
Smoking,* pack-years	0 (0, 23.0)	0 (0, 50.8)	0.718
Asthma family history, Y/N	15/47	41/82	0.202
Asthma duration, yrs	4.9±7.1	10.1±13.2	0.029
Dyspnea VAS	17.37±22.01	19.95±23.07	0.467
Borg score	1.06±1.13	1.44±1.44	0.070
Chest tightness and VAS	16.53±18.42	18.11±22.47	0.545
Cough and VAS	13.10±21.94	14.19±20.54	0.322
Wheezing and VAS	13.13±20.01	13.93±21.10	0.784
GINA treatment steps			
Steps 1, 2, 3, 4, and 5	19/7/11/25	9/14/34/66	<0.001
ACQ	1.18±0.86	1.50±0.89	0.0213
AQLQ	5.29±0.88	5.02±0.97	0.061
Activity limitations	5.55±0.88	5.25±1.03	0.044
Asthma symptoms	5.01±0.98	4.74±1.08	0.101
Emotional distress	5.32±1.16	5.36±1.30	0.588
Environmental stimuli	5.40±1.32	4.78±1.26	0.002
FEV1, L	3.29±0.69	2.54±0.53	<0.001
FEV1, % predicted	104.24±11.69	90.47±7.00	<0.001
FEF25-75, % predicted	84.58±16.55	48.34±10.29	<0.001
FEV1/FVC, %	84.59±5.29	72.85±6.22	<0.001
PD20, mg	0.77±0.67	0.68±0.69	0.276
Asthma medications Aminophylline, Y/N	9/53	31/92	0.096
SABA, Y/N	10/52	19/104	0.904
ICs plus LABA, Y/N	11/51	16/107	0.389
Systemic corticosteroids, Y/N	3/59	7/116	0.809
Asthma events in the past year Exacerbations*	1 (1, 2)	1 (1, 2)	0.106
Emergency visits*	0 (0, 0)	0 (0, 0)	0.537
Hospitalizations*	0 (0, 0)	0 (0, 0)	0.552

ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; ICs, inhaled corticosteroids; PD20 = provocative dose of methacholine required to achieve a 20% fall in FEV1; SABA, short-acting betaagonists; SAD, small airway dysfunction; VAS, visual analog scale; Y/N, yes/no; FEF25−75, forced expiratory flow between 25 and 75%; LABA, long-acting beta-agonists; GINA, Global Initiative for Asthma. * Variables are expressed as median and interquartile range.

MCT in comparison to those without SAD (online suppl. Table 1; see www.karger.com/doi/10.1159/0005515328 for all online suppl. material). Expected increases in respiratory symptoms were successfully produced by the MCT (online suppl. Table 1). As a result, ΔVAS for total symptoms including dyspnea, wheezing, chest tightness,

and cough (p = 0.0294) (Fig. 1) and ΔVAS/FEF25–75 representing an index of total symptoms (p = 0.0006) (Fig. 2) in SAD were elevated significantly. Patients with SAD had significantly increased ΔVAS for dyspnea (p = 0.027) and chest tightness (p = 0.032) but not the ΔBorg score, wheezing and cough, in comparison to patients without SAD (Fig. 1). The ΔBorg for dyspnea (p = 0.0294) and ΔVAS for dyspnea (p = 0.0009), chest tightness (p = 0.0052), and wheezing (p = 0.0539) against ΔFEF25–75, as indices of symptoms in patients with SAD, were also statistically elevated compared to those without small airway impairment (Fig. 2). We further explored the impacts of SAD on symptom perception during bronchoconstriction. Multiple logistic

Fig. 1. Changes in respiratory symptoms between individuals with SAD and without SAD (non-SAD) in study I. Variables are expressed as median and interquartile range with 5–95 percentile. SAD, small airway dysfunction;
VAS, visual analog scale.

regression analysis suggested that SAD was associated with ΔBorg for dyspnea (OR = 2.51, 95% CI = [1.05, 6.02], p = 0.039), ΔVAS for dyspnea (OR = 4.25, 95% CI = [1.48, 12.24], p = 0.007) and ΔBorg/ΔFEF25–75 (OR = 2.46, 95% CI = [1.02, 5.89], p = 0.044), and ΔVAS/ΔFEF25–75 in dyspnea (OR = 4.09, 95% CI = [1.42, 11.79], p = 0.009) (Fig. 3).

SAD and Airway Inflammation

As for the inflammatory mediators in induced sputum supernatants, IFN-γ (1.95 [1.63, 2.54] vs. 1.67 [1.38, 1.85] pg/mL, p = 0.004) and IL-1β (18.14 [8.39, 46.59] vs. 9.82 [5.38, 25.09] pg/mL, p = 0.047) in SAD patients with asthma were significantly increased compared with non-SAD patients with asthma. There was also a trend

Fig. 2. Slope of ΔBorg for dyspnea and ΔVAS for respiratory symptoms against ΔFEF25–75 between individuals with SAD and without SAD (non-SAD) in study I. SAD, small airway dysfunction; FEF25−75, forced expiratory flow between 25 and 75%; VAS, visual analog scale.

Fig. 3. Logistic regression models for relationships between SAD and dyspnea in study I. Adjusted for age, sex, BMI, smoking, asthma family history, asthma duration, and baseline Borg in dyspnea or VAS score of symptoms and FEV1 %predicted before MCT. SAD, small airway dysfunction; VAS, visual analog scale; MCT, methacholine challenge testing.

toward IL-8 elevation in the SAD patients with asthma IL-17A, and TNF-α between the 2 groups (all p > 0.05) compared with non-SAD patients with asthma, but it (Table 2). did not reach statistical significance (p = 0.060). There Correlations of SAD with airway inflammatory prowere no significant differences in IL-4, IL-5, IL-6, IL-13, files indicated that either FEF25–75 (L/s) or FEF25–75 (%

Table 2. Inflammatory profiles and phenotypes in patients with asthma grouped by SAD in study II

Variable	SAD	Non-SAD	p value
N	86	61	
Age, yrs	48.95±13.67	38.35±11.98	<0.001
Gender, female (%)	48 (55.8)	37 (60.7)	0.558
BMI	24.27±3.54	22.69±3.03	0.005
Smoking, pack-years	0.0 (0.0, 8.5)	0.0 (0.0, 0.85)	0.224

Baseline ICs, Y/N	35 (40.7)	28 (45.9)	0.530
BDP, µg	0 (0, 400)	0 (0, 400)	0.253
Sputum			
Eosinophil, %	0.00 (0.00, 1.00)	0.00 (0.00, 1.00)	0.794
Neutrophil, %	39.75 (14.75, 57.25)	17.00 (7.00, 39.25)	0.045
Lymphocyte, %	1.00 (0.25, 2.00)	0.75 (0.25, 1.50)	0.248
Macrophage, %	56.75 (28.00, 81.00)	79.22 (60.00, 91.75)	0.016
FENO, ppb	38 (18, 67)	28 (22, 56)	0.931
IgE, IU/mL	146.00 (39.66, 285.51)	129.99 (40.73, 346.25)	0.954
Cytokines in sputum IFN-γ, pg/mL	1.95 (1.63, 2.54)	1.67 (1.38, 1.85)	0.004
IL-13, pg/mL	2.87 (1.96, 4.70)	2.60 (1.76, 3.70)	0.241
IL-17, pg/mL	2.56 (1.59, 4.48)	2.19 (1.75, 3.53)	0.478
IL-1β, pg/mL	18.14 (8.39, 46.59)	9.82 (5.38, 25.09)	0.047
IL-4, pg/mL	23.93 (2.25, 108.18)	10.30 (2.25, 48.38)	0.117
IL-5, pg/mL	1.23 (0.90, 2.55)	1.32 (0.86, 4.73)	0.690
IL-6, pg/mL	19.57 (5.79, 55.56)	18.42 (5.31, 42.52)	0.732
IL-8, pg/mL	1,764 (598.14, 3,925.00)	1,194 (645.62, 2,017.00)	0.060
TNF-α, pg/mL	12.94 (3.69, 42.66)	9.94 (4.22, 15.61)	0.238

SAD, small airway dysfunction; FENO, fractional exhaled nitric oxide; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; IC, inhaled corticosteroid.

pred) negatively correlated to IFN-γ (r = −0.243, p = 0.018 and r = −0.222, p = 0.032), IL-8 (r = −0.254, p = 0.013 and r = −0.219, p = 0.034), and sputum macrophages (%) (r = 0.257, p = 0.017 and r = 0.215, p = 0.047). The percentage of neutrophils in induced sputum was associated with FEF25–75 (L/s) (r = −0.23, p = 0.033) but not FEF25–75 (% pred) (r = −0.178, p = 0.101) (Fig. 4). However, there were no statistically significant correlations between IL-1β and FEF25-75 (online suppl. Table 2). After adjusting for age, gender, BMI, smoking, FEV1% pred, and IC dose, there were still relationships between FEF25–75 (L/s) and neutrophil percentage in sputum (r = −0.224, p = 0.043), IFN-γ (r = −0.228, p = 0.031), IL-8 (r = −0.251, p = 0.017), and macrophage percentage in sputum (r = 0.242, p = 0.028). Meanwhile, there were still relationships between FEF25–75 (% predicted) and IFN-γ (r = −0.213, p = 0.044), IL-8 (r = −0.217, p = 0.040), and there was a trend relationship between FEF25–75 (% predicted) and macrophage percentage in sputum (r = 0.203, p = 0.067) (online suppl. Table 2).

Treatment Responsiveness Heterogeneity across SAD and Non-SAD Subjects

In study III, a total of 65 subjects completed the 4-week fixed treatments. Twenty-six subjects with non-SAD had a greater change in ACQ or ACT scores than subjects with SAD (n = 39) (−0.5 [−1.04, 0.00] vs. −0.17 [−0.50, 0.21], p = 0.045 for ACQ, and 3.65 ± 4.08 vs. 1.36 ± 4.00, p = 0.028 for ACT). More patients had clinically significant changes in ACQ (defined as a change of greater than 0.5) in non-SAD asthma patients compared with SAD asthma patients (53.8 vs. 25.6%, p = 0.021), but it was only a trend in ACT (a change of greater than 3 was considered clinically important) (35.9 vs. 57.7%, p = 0.083). In addition, the treatment response in terms of ΔFEV1 (% pred) was comparable between the SAD and non-SAD groups (p > 0.05) (Table 3). Multivariable regression analysis showed that either baseline FEF25–75 (L/s) or FEF25–75 (% pred) predicted minimal clinical improvement in ACQ (OR = 2.520, 95%

CI = [1.149, 5.524], p = 0.021 and OR = 1.040, 95% CI = [1.006, 1.075], p = 0.022) and ACT (OR = 2.192, 95% CI = [1.083, 4.437], p = 0.027 and OR = 1.034, 95%

Fig. 4. Correlations between inflammatory profiles and SAD in study II. SAD, small airway dysfunction; IFN, interferon; IL, interleukin; FEF25−75, forced expiratory flow between 25 and 75%.

Table 3. Treatment responsiveness across SAD and non-SAD subjects in study III

Variable	SAD	Non-SAD	p value
N	39	26	
Age, yrs	46.79±13.33	35.73±12.99	0.002
Gender, female (%)	29 (74.4)	17 (65.4)	0.436
BMI	22.55±3.84	21.88±2.91	0.451
Smoking, pack-years	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.615
Baseline ICs, Y/N	11/28	10/16	0.386
BDP, μg Spirometry	0 (0, 400)	0 (0, 400)	0.525
ΔFEF25−75, L/s	0.08±0.61	−0.12±0.62	0.209
ΔFEF25−75, %	6.00 (−23.53, 29.94)	−2.04 (−15.48, 8.95)	0.538
ΔFEV1, L	−0.03 (−0.16, 0.17)	0.03 (−0.12, 0.19)	0.560
ΔFEV1, %	−1.13 (−7.31, 7.93)	0.99 (−3.61, 5.82)	0.630
ΔFEV1 ≥ 12%, n (%)	6 (15.4)	1 (3.8)	0.228
Response in asthma control			
ΔACQ			
ΔACQ ≥ 0.5, n (%)			
ΔACT			
ΔACT ≥ 3.0, n (%)			

Δ, change from baseline; FEF25−75, forced expiratory flow between 25 and 75%; ACQ, Asthma Control Questionnaire; ACT, Asthma Control Test; SAD, small airway dysfunction; IC, inhaled corticosteroid.

Table 4. Multivariable regression analysis for the influence of SAD on treatment response in study III

Outcome	Baseline FEF25−75, L/s		Baseline FEF25−75 % predicted		
	β	95% CI	β	95% CI	value
ΔACQ ≥ 0.5*					

SAD, small airway dysfunction; FEF25−75, forced expiratory flow between 25 and 75%; ACQ, Asthma Control Questionnaire; ACT, Asthma Control Test; LTRA, leukotriene receptor antagonist; Δ, change from baseline.

* Adjusted for age, gender, BMI, smoking, FEV1% predicted, IC dose, and the use of LTRA at baseline.

CI = [1.003, 1.066], p = 0.032) after adjusting for age, gender, BMI, smoking, FEV1% pred, IC dose, and the use of LTRA at baseline (Table 4).

Sensitivity Analysis

Finally, we did further sensitivity analyses of primary outcomes for using the LLN of FEF25–75% pred as the cutoff of SAD, which confirmed and strengthened our findings that SAD was associated with more significantly symptom perception, worse inflammation, and poor responsiveness to therapy. Based on the LLN as the reference definition of SAD [40, 41], 65% predicted FEF25–75% had a sensitivity of 97.37% and a specificity of 83.10% in identifying SAD, in which the area of receiver operating characteristic curve was 0.979 (online suppl. Table 3). In study I, patients with SAD with FEF25–75% < LLN (LLNdefined SAD) (n = 114) had significantly increased ΔVAS for dyspnea (p = 0.027), chest tightness (p = 0.032), and total symptoms (p = 0.029) in comparison with non-SAD (LLN-defined non-SAD) (n = 71). The slope of ΔVAS/
ΔFEF25–75 for dyspnea (p = 0.007), chest tightness (p = 0.013), and total symptoms (p = 0.010) in patients with LLN-defined SAD were statistically elevated compared to LLN-defined non-SAD (online suppl. Table 4). As for the inflammatory mediators in induced sputum supernatants in study II, IFN-γ (1.96 [1.68, 2.68] vs. 1.74 [1.41, 2.01] pg/ mL, p = 0.010) and IL-17 (3.39 [2.01, 5.08] vs. 2.10 [1.59, 3.62] pg/mL, p = 0.027) in LLN-defined SAD patients with asthma (n = 60) were significantly increased in comparison with LLN-defined non-SAD patients with asthma (n = 87). However, no differences were found in terms of neutrophil percentage in sputum and IL-4, IL-5, IL-6, IL13, IL-1β, and TNF-α between groups (online suppl. Table
5). Similarly, in study III, subjects with LLN-defined nonSAD (n = 35) had a greater improvement of ACT scores than subjects with LLN-defined SAD (n = 30) (3.20 ± 4.41 vs. 1.20 ± 3.61, p = 0.049). More patients had clinically significant changes in ACQ (defined as ΔACQ ≥ 0.5) and ACT (defined as ΔACT ≥ 3) in LLN-defined non-SAD asthma patients compared with LLN-defined SAD asthma patients (51.4 vs. 20%, p = 0.009 for ACQ; 57.1 vs. 30%, p = 0.028 for ACT) (online suppl. Table 6).

Discussion

To the best of our knowledge, this is the first study based on an a priori hypothesis to explore the effects of SAD on perceived respiratory symptoms in asthma during bronchoconstriction and the direct effect of SAD on treatment responses. We have concurrently elaborated on the association between SAD and airway inflammation profiles. As a result, we found direct evidence that SAD predisposed patients to greater intensity of dyspnea and chest tightness. Further, asthma patients with SAD were characterized by type 1 or non-type 2 inflammation and poor responsiveness to therapy, which indicated potential implications for precision medicine treatment in asthma with SAD.

Recently, increasing evidence indicated that SAD was associated with asthma symptoms and control [6, 16, 20], which is in agreement with our results showing that individuals with SAD were predominately older, female, with longer asthma duration, with more severe asthma, and poorer asthma control and quality of life in comparison to those without SAD. Meanwhile, we have found that BMI in the SAD group was higher than that in the nonSAD group. However, the mean values of BMI in both groups were within the normal range, and there was no difference between groups in terms of obesity, which indicated that the relationship between BMI and SAD remained to be further explored. Interestingly, we also observed that compared with the non-SAD group, the SAD group had a smaller decrease in FEF25–75 and increased dyspnea intensity and chest tightness during the MCT.

Steeper slopes for dyspnea and chest tightness against ΔFEF25–75, as an index of the intensity of symptom changes, were found in patients with SAD compared to those in the non-SAD group. Furthermore, smaller decreases in FEF25–75 may be a sign of fixed

obstruction in the small airway, which may lead to a more sensitive perception for asthma symptoms [42].

Several other studies have also observed symptoms in chronic airway diseases or asthma induced by Mch [43, 44]. In the study by Mansur et al. [43], a significant correlation was observed between methacholine-induced dyspnea and the change in resistance of 5 Hz (R5) and reactance at 5 Hz (X5) but not with FEV1. However, they did not explore the relationship between the resistance at 5 Hz minus resistance at 20 Hz (R5–R20) and methacholine-induced symptoms. In a retrospective study based on MCT among subjects with persistent respiratory symptoms and normal chest radiograph [44], Segal et al. [44] found disparate behavior of proximal airway resistance (FEV1 and resistance at 20 Hz [R20]) and distal airway heterogeneity (R5-R20 and area of reactance [AX]); furthermore, distal airway reactivity may be associated with methacholine-induced symptoms, despite the absence of FEV1 changes. Our study further validated the effects of SAD on methacholine-induced symptoms, and we found that SAD predisposed patients to greater dyspnea intensity and chest tightness during bronchoconstriction in asthma, which could account for poor asthma control. Meanwhile, SAD originated from distal airway inflammation, which was characterized by a steep slope as an index of symptoms [45].

As our results have shown that type 1 or non-type 2 inflammation was enhanced as elevated levels of IFN-γ in asthma patients with SAD, which may also account for treatment responsiveness heterogeneity across the SAD and non-SAD subjects. It recently has indicated IFN-γ involving a possible trigger of GC insensitivity or severe asthma [46]. Notably, it had inconsistent findings in terms of inflammatory mediators such as sputum neutrophils, IL-1β, and IL-17 between primary analysis and sensitivity analysis, which would partly be explained by small sample size. Furthermore, the correlation analysis indicated that FEF25–75 (L/s) or % pred) was associated with worse airway inflammation, especially enhanced type 1 or non-type 2 inflammation such as IFN-γ and IL-8. Some other studies supported the role of type 1 or non-type 2 inflammation in the pathogenesis of SAD [24, 47, 48]. Farah and his colleagues found that neutrophilic airway inflammation is associated with dysfunction of the peripheral, diffusion-dependent acinar airways in stable asthma [24]. In addition, SAD was also found to be associated with neutrophilic inflammation in bronchial biopsies and BAL in patients with COPD and cystic fibrosis [47, 48]. On the contrary, Riley et al. [49] found that SAD correlates to eosinophilic inflammation, which was not in line with our results. The difference would be explained by a different population with asthma as follows. First, the study setting and race could be possible factors explaining this difference in inflammatory feature. Second, we excluded those patients with FEV1 <80% predicted based on the small airway asthma phenotype [7] in our study. Third, we did not exclude current smokers, which would influence airway inflammation [50], although no difference was found between non-SAD and SAD groups.

Therefore, the function of peripheral airways in asthma is potentially complex and affected by different airway inflammation profiles, which needs to be further studied. Anyway, patients with asthma with preserved FEV1 might be further endotyped according to the involvement of SAD with targeted therapeutic implications as its features of worse airway inflammation.

In the present study, we defined SAD based on
FEF25–75%, with a cutoff of 65% in the primary analysis. Theoretically, the LLNs is the standard to define SAD in terms of FEF25–75% [40, 41]. LLN as one of lung function parameters is taken to be equal to more than 1.64 standard deviation below the predicted

level (the 5th percentile) of a healthy, nonsmoking population, which should improve accuracy and confidence to the diagnostic approach, but this recommendation has not been accepted by global guidelines [51–53]. In fact, simple and commonly used cutoff such as an FEV1/FVC <0.70 to indicate airflow obstruction, or assuming FEV1 <80% of predicted to be abnormal, was widely used in clinical practice. Similarly, for clinical convenience, FEF25–75 <65% of the predicted value is defined to be abnormal by Chinese guidelines for lung function testing, which has been widely applied in clinical practice in the Chinese population [54] and other populations worldwide [29–31]. In addition, based on the LLN as the reference definition of SAD, 65% of the predicted of
FEF25–75 had a sensitivity of 97.37% and a specificity of 83.10% in identifying SAD, with the area of receiver operating characteristic curve 0.979, indicating an excellent accuracy. Anyway, we performed sensitivity analysis to strengthen our findings by identifying SAD using FEF25–75% < LLN. Our study has several limitations that need to be addressed. First, FEF25–75 is not the gold standard to diagnose SAD, although we performed sensitivity analysis to strengthen our findings by identifying SAD using FEF25–75% < LLN across all parts of this study. Second, univariate correlations between inflammatory profiles and SAD were tested statistically without a multiplicity adjustment, which are needed to be further validated. Third, individuals with normal FEV1 may limit the generalizability of the results because of lower air trapping and the relatively mild severity of asthma. Fourth, patients in study III were not all treatment-naive prior to the baseline evaluation, which may confound the treatment effect. As all of the patients included in the study were suboptimally managed and severely uncontrolled, we started the patients with medium-dose IC-LABA + LTRA according to the GINA-preferred initial treatment. However, all of the subjects received a one-week washout period to prevent the carryover effect, and considering the patients' need to treatment to asthma control and the medications at baseline were adjusted in the multiple regression models when the treatment responsiveness was assessed.

In conclusion, we provide supportive evidence for the contribution of SAD to the clinical expression of asthma. This study suggests that SAD in asthma independently predicts worsening symptoms of dyspnea and chest tightness and patients with SAD are predisposed to greater dyspnea intensity and chest tightness during bronchoconstriction, which contribute to poor asthma control. Furthermore, asthma with SAD is associated with worse airway inflammation in patients, especially type 1 or nontype 2 inflammation that may account for poor responsiveness to therapy. This study partially validates the small airway asthma phenotype, which indicates that the possibility of SAD should be considered in the complete spectrum of GINA treatment steps.

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Statement of Ethics

The Institutional Review Board at the West China Hospital approved all protocols (Nos. 2014-30 and 2013-65), and written informed consent was obtained from all subjects.

Conflict of Interest Statement

Dr. G. Wang has received a China AstraZeneca Lung Foundation grant. The other authors declare that they have no competing interests to declare.

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Author Contributions

G.W. conceived the study, performed the data interpretation and manuscript revision, and took accountability for all aspects of the work. Y.L. and L.Z. planned the work, carried out the data analysis and interpretation, and drafted the manuscript. H.L.L., B.M.L., J.W., X.Z., and H.P.Z. conducted the participant recruitment and project for disciplines of excellence Clinical Research Incubation participated in data analysis and interpretation. Z.H.C., M.X., L.W., and B.G.O. interpreted the results and contributed to the manu-

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