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Original Article

Risk factors related to persistent airflow obstruction in severe asthma in Chinese Han population

Lanlan Zhang[1*], Wenjuan Yang[1*], Qiao Zhou[2], Gang Wang[3], Chuntao Liu[1]

[1]Department of Respiration Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan, China; [2]Pathology of Respiration Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan, China; [3]Department of Integration of Traditional Chinese and Western Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan, China. [*]Equal contributors.

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Abstract: Objective: To explore the significance of assessing persistent airway obstruction (PAO) in asthma patients by airway wall remodeling with bronchoscopy, high-resolution computed tomography (HRCT), and biological markers in the induced sputum and serum, exhaled nitric oxide (FENO), and lung function. Methods: The study was conducted in 119 patients with PAO and 125 patients with reversible airway obstruction (RAO). Endobronchial biopsy specimens were analyzed for airway smooth muscle (ASM) area, and reticular basement membrane (RBM) thickness. Airway thickness was also measured by HRCT scanning. Levels of matrix metalloproteases-9 (MMP-9), metalloproteinase 33 (ADAM33), and vascular endothelial growth factor (VEGF) were measured in the induced sputum and serum by enzyme linked immunosorbent assay. Result: PAO was associated with longer disease duration, absence of atopy and rhinitis, and larger ASM area (SMA%) (15.83%±2.32% [n=9] vs. 8.0%±1.68% [n=7], *P*=0.02), thicker RBM (16.27±2.32 μm [n=9] vs. 8.71±2.41 μm [n=7], *P*=0.042); No differences in any of the biomarker molecules measured in airway thickness in HRCT, sputum and blood individually between groups were found. Conclusion: Severe asthma patients with longer disease duration and the absence of atopy and rhinitis are more likely to develop PAO in Chines Han population. PAO patients have increased ASM area and RBM thickness appear to be valuable in the evaluation of airway remodeling in asthma patients in Chinese Han population.

Keywords: Bronchoscopy, high-resolution computed tomography (HRCT), persistent airway obstruction (PAO), reversible airway obstruction (RAO)

Introduction

In spite of accounting for only 5% to 10% of the total asthma cases, severe asthma leads to a disproportional utilization of the health care resources [1]. Asthma has been traditionally considered as a reversible disease of airflow obstruction. In recent years, however, persistent airway obstruction (PAO) has been acknowledged. For instance, Vonk et al. [2] examined 228 adults with a history of asthma at the ages ranging from 13 to 44 years at baseline and found that after 26 years of follow up, 41% of them had no airway obstruction (NAO), 43% had reversible airway obstruction (RAO) and 16% had PAO. However, the pathological basis of irreversible airway obstruction (IAO) is currently unknown, and whether or not IAO is associated with any distinct pattern of airway remodeling has not been elucidated in Chinese Han population, the largest population in the world.

Some features of airway remodeling, particularly the increased airway smooth muscle (ASM), have been associated with severity of asthma [3, 4]. High-resolution computed tomography (HRCT) has been used as a method for noninvasive evaluation of airways, and it has the potential to evaluate airways in patients with obstructive pulmonary disease [5]. Increased matrix metalloproteases-9 (MMP-9), metalloproteinase 33 (ADAM33), and vascular endothelial growth factor (VEGF) levels have been known to be correlated with the severity of airflow obstruction in asthma [6-8]. All of these markers described above may be used to examine the structural changes in the large or small airways in asthma. We hypothesized that there existed the key risk factors related to PAO

Table 1. Criteria for inclusion of asthma severity

Major criteria (all required)	Minor criteria (one of them)
Treatment with daily oral steroids for more than 50% of the previous 12 months	Need for daily short-acting beta-agonist,
Treatment with moderate- to high-dose ICS and an LABA combination inhaler over the previous 12 months	1 or more emergency care visits in the last 12 months
Patients who underwent at least 3 pulmonary function tests under stable conditions	3 or more steroid bursts in the last 12 months
Patients who were adequately treated and adherent to prescribed medications	Prompt deterioration with 25% or lower dose reduction of oral corticosteroids
-	Near-fatal asthma event in the last 3 years

in severe asthma in Chinese Han population. To test this hypothesis, we conducted a consecutive observational study of adult patients with severe asthma, aiming to investigate the correlations of different types of asthma (PAO and RAO) with the severity of airway remodeling evaluated by bronchoscopy, HRCT, exhaled nitric oxide (FENO), and cytokines in induced sputum and serum by enzyme linked immunosorbent assay (ELISA).

Method

Subjects

This study was a prospective, observational, and 1-year study of patients with severe asthma in the Chinese Han population. The study group comprised of the randomly selected asthma patients who visited outpatient clinics in West China Hospital from January 2011 to March 2013. The study protocol was approved by the Ethics Committee of the West China Hospital and written informed consent was obtained from each of the recruited patients and the healthy participants. The study subjects were 18 years or older. Asthma was diagnosed by demonstration of airway reversibility or bronchial hyperresponsiveness (BHR). Airway reversibility was consisted as an improvement in FEV1of at least 12% post-bronchodilator (200 mcg of albuterol by means of a metered-dose inhaler), or 20% or more over time or after corticosteroid treatment. BHR was defined as PD_{20} (provocation concentration that caused a decrease in FEV1 of 20% of methacholine ≤16 mg/mL [9].

Inclusion and exclusion criteria for the patients recruited in this study

Inclusion criteria at were as follows: severe asthma patients (see the **Table 1**): severe asthma was defined in accordance with the American Thoracic Society (ATS) workshop on refractory asthma [10]. Symptoms of asthma were evaluated according to current ATS criteria [11]. Exclusion criteria were the patients with specific respiratory disease and/or any other seriously interfering diseases and the patients who had to be in a stable condition without an exacerbation in the previous 6 weeks. Subjects were followed up monthly for at least 12 months.

Definition of fixed airway obstruction and reversible airway obstruction

Patients with severe asthma were classified into two groups, one group with PAO and the other one with RAO, based on the results of their pulmonary function tests. PAO was defined as a postbronchodilator FEV1/FVC and FEV1% ratio <0.7 on all of three pulmonary function tests despite the use of high-dose inhaled corticosteroids (ICS) and long-acting b2-agonists (LABA). The available follow-up was at month 12; RAO was defined as a postbronchodilator FEV1/FVC >0.7, inclusive, at both baseline and first annual follow-up assessment.

Procedures

Spirometry was performed according to ATS standards. Allergy skin prick or UniCAP100 (CPA) tests were performed with the commercial extracts from common allergens. Sputum was induced by using inhalations of increasing concentrations (3, 4, and 5%) of hypertonic saline. The levels of FENO were measured using a chemiluminescence analyzer (NiOX; Aerocrine, Stockholm, Sweden) before any forced expiratory maneuvers according to current guidelines at an exhaled flow rate of 50 ml/second [12]. Histologic image analysis was performed with the Image Pro-Plus 4.0 system (Media Cybernetics, Silver Spring, MD, USA).

Table 2. Characteristics of reversible airway obstruction and persistent airway obstruction patients

Characteristics	PAO	RAO	*P* value
N	117	125	-
M (F). no.[$]	40 (77)	38 (87)	0.538
Age (year)	48.80±10.83	41.66±11.68	0.000
BMI[*]	23.15±5.20	22.57±3.72	0.324
Present smoker/Ex-smoker	18/117	17/125	0.583
Untreated period (yr)[*]	12.09±13.26	19.38±16.22	0.001
Disease duration (yr)	19.38±16.22	12.06±13.26	0.000
Allergic rhinitis. no.[$]	34/117	63/125	0.000
Family history of asthma. no.[$]	43	30	0.043
Exacerbations in past 2 year[*]	3.01±9.55	1.30±3.0	0.082
Hospital in past 2 year[*]	2.18±4.25	0.97±3.24	0.07
Inhaled long-actingb2-agonist plus ICS, no. (%)			
Systemic steroids, no. (%)			

Values are presented as means ± SDs unless otherwise stated. F, Female; M, male; BMI, body mass index; ACT, asthma control test; ICS, Inhaled corticosteroids; Atopy, referred to positive skin prick test response. [*]Wilcoxon rank sum test; [$]Chi-square test.

Images were recorded on a CCD color video camera (Sony, Japan) mounted on a conventional light microscope (Olympus Optical Co, Tokyo, Japan). The program was used to analyze the percentage of intact epithelium, ASM and RBM thickness.

Bronchoscopy

During bronchoscopy, 16 endobronchial biopsies were done at various segmental and subsegmental carinae of the right lung. Tissues were fixed in formaldehyde and embedded in paraffin in random orientation before cutting. All slides were screened for adequacy of the section before staining in a blinded fashion as to the source of subjects. Staining of the section slides was performed by using a peroxidase-based method. ASM was detected with the monoclonal anti-SMA antibody clone 1A4 (Sigma, St Louis, MO, USA) by using the DAB method (DakoCytomation, Inc, Glostrup, Denmark). RBM thickness was measured on hematoxylin and eosin (HE)-stained slides.

High-resolution computed tomography (HRCT) scanning

HRCT scans of the chest were done as previously reported [13].

Measurement of the levels of MMP-9, VEGF, and ADAM33 in serum and the induced sputum

Levels of MMP-9, VEGF and ADAM33 in serum and the induced sputum were determined by enzyme immunoassays according to the manufacturer's protocol .Briefly, venous blood samples were collected from all participants. Five ml of blood sample from each participant was centrifuged at 2000 rpm and 4°C, sub-packaged, and cryopreserved at -80°C. The serum levels of MMP-9, VEGF, and ADAM33 were measured by sandwich enzyme linked immunosorbent assay (ELISA) using the commercially available ELISA kits for MMP-9, VEGF and ADAMM33. Sputum plugs arising from the lower respiratory tract were selected for sputum cell counts. The levels of MMP-9, VEGF, and ADAM33 were measured in duplicate using the corresponding kits, respectively. Sputum induction and processing were performed according to Pavord et al. [14]. Briefly, prior to sputum induction, in order to decrease bronchial constriction during the induction procedure, asthmatic patients were advised to inhale 400 mg of salbutamol. Sputum was firstly induced by inhalation of nebulized hypertonic saline solution (3%) for 15 min, and then patients were encouraged to expectorate sputum. All adequate plugs of sputum were separated from saliva by centrifuge at 2000 rpm for 10 min. The supernatant was saved and mixed with an equal volume (to DTT) of Dulbecco's phosphate buffered saline (D-PBS). After centrifugation, the proportions of differential types of cells including eosinophils, macrophages, lymphocytes, and neutrophils in sputum supernatant were determined by counting a total of 400 non-squamous cells. The counts of these types of cells were then expressed in the specimens stained.

Table 3. Pulmonary function, ACT and FENO tests of patients with severe asthma

Characteristics	PAO		RAO	
N	117	97	125	104
FEV1 (L)	Baseline	End of study	Baseline	End of study
	1.22±0.51[&]	1.54±0.66[#]	2.41±0.71	2.89±0.58
FEV1 after bronchodilator (L)	Baseline	End of study	Baseline	End of study
	1.37±0.51[&]	-	1.87±0.81	-
FEV1 (%)	Baseline	End of study	Baseline	End of study
	46.72±16.33[&]	57.52±17.86[#]	83.55± 17.09	90.25±9.06
FEV1 after bronchodilator (% predicted)	Baseline	End of study	Baseline	End of study
	58.12±25.04	-	67.46±17.25	-
FEV/FVC%	Baseline	End of study	Baseline	End of study
	51.81±12.43[&]	56.17±17.30[#]	73.88±11.97	79.36±9.29
FEV1/FVC ratio after bronchodilator (%)	Baseline	End of study	Baseline	End of study
	51±11.37[&]	-	70.25±19.44	-
PEF (ml)	Baseline	End of study	Baseline	End of study
	183.03±100.76[&]	222.70±106.92[#]	333.92±123.10	400.67±109.27
FENO (pbb)[*]	Baseline	End of study	Baseline	End of study
	46.69±38.80[&]	35.30±12.09	60.95±49.25	66.42±36.09
ACT score[*]	Baseline	End of study	Baseline	End of study
	16.09±8.47	21.33±3.30[#]	17.24±4.30	22.67±2.45

Values are presented as means ± SDs unless otherwise stated. FVC, forced vital capacity. [*]Wilcoxon rank sum test; Chi-square test. [&]*P*<.05 versus baseline of with reversible airflow obstruction. [#]*P*<.05 versus end of study with reversible airflow obstruction.

Statistical analysis

Data were analyzed with t-Student test and Wilcoxon rank sum test. Clinical data were expressed as means ± SDs. Remodeling and biomarker data were expressed as means ± SDs or SEMs. Pearson correlation coefficients were calculated with SPSS. Multiple logistic regression analysis was used to compute the adjusted OR (for possible confounding factors) and to assess the independence of the relation between each factor and the presence of severe PAO. The following contrasts were used: ex- or current smoker versus never smoker, atopic versus non-atopic, age of asthma onset (≥18 versus <18 years (yrs), disease duration (≥11 versus <11 yrs); untreated period (≥10 versus <10 yrs), FEV1 reversibility (≥15% versus <15%), exhaled NO (≥39 ppb versus <39 ppb), presence versus absence of bronchial wall thickening on HRCT. a *P*-value <0.05 was considered statistically significant.

Result

Of a total of 242 patients with severe asthma recruited for this study, there were 117 patients with PAO and 125 patients with RAO. The demographic characteristics of these patients were listed in **Table 2**. Patients with PAO were significantly older than those with RAO (48.80±10.83 yrs vs 41.66±11.68 yrs, *P*=0.000). In addition, patients with PAO were more likely to have a longer untreated period (12.09±13.26 yrs vs 19.38±16.22 yrs, *P*=0.001) and a significantly longer disease duration (19.38±16.22 yrs vs 12.06±13.26 yrs, *P*=0.000). Allergic rhinitis and family history of asthma were significantly less common in patients with PAO than in patients with RAO (29% vs 50%, *P*=0.000), (37% vs 24%, *P*=0.043), respectively. There were no significant between-group differences in male, numbers of emergency department visits and admissions in recent 2 years due to acute asthma exacerbation, BMI (kg/m^2), present smoker/ex-smoker, status and history of asthma exacerbation in recent 2 years (**Table 2**). The patients with PAO and RAO had prebronchodilator FEV1 and an increasing ACT over 12 months, had a decreasing FENO in PAO group (**Table 3**).

Risk factors

Multivariate analysis identified several independent factors associated with PAO (**Table 2**).

Table 4. Risk factors of irreversible bronchoconstriction assessed by logistic multivariate regression (after reduction of insignificant variables)

Characteristics	Adjusted odds ratio (95% confidence interval)	*P*-value	Unadjusted odds ratio# (95% confidence interval)	*P*-value
Sputum eosinophils >5%	1.65 (0.59, 4.63)	0.908	1.78 (0.64, 4.92)	0.27
Exhaled NO >39 ppb	0.55 (0.28, 1.1)	0.077	0.573 (0.29, 1.12)	0.106
Reversibility of FEV1 >15%	1.11 (0.33, 3.77)	0.531	1.04 (0.31, 3.47)	0.945
Sputum neutrophils >64%	0.76 (0.27, 2.22)	0.627	0.73 (0.25, 2.10)	0.557
Present smoker/Ex-smoker	0.93 (0.45, 1.91)	0.84	0.95 (0.47, 1.93)	0.889
Disease during >11 yr	1.96 (1.16, 3.3)	0.11	1.95 (1.16, 3.29)	0.011
Untreated period >10 yr	1.24 (0.46, 3.31)	0.67	1.92 (1.13, 3.28)	0.016
Atopic	0.20 (0.07, 0.54)	0.002	0.252 (0.098, 0.648)	0.004
Allergic rhinitis	0.51 (0.30, 0.88)	0.015	0.45 (0.22, 0.94)	0.033
Male	1.13 (0.65, 1.97)	0.65	1.12 (0.66, 1.91)	0.666
Family history of asthma	1.28 (0.74, 2.22)	0.379	1.21 (0.50, 1.55)	0.65
A college education or advanced degree	0.44 (0.17, 1.33)	0.89	0.43 (0.17, 1.08)	0.075
Bronchial wall thickening >65%	0.53 (0.72, 3.92)	0.536	0.844 (0.143, 4.97)	0.851

Data are presented as OR (95% CI). #: adjusted OR for disease duration, male.

Disease duration was the most importantly independent factor associated with FAO, with an adjusted odds ratio (OR) for disease duration of 1.96 [95% confidence interval (CI), 1.16 to 3.3]. In addition, FAO was associated with atopy asthma (adjusted OR, 0.2; 95% CI, 0.07 to 0.54) and allergic rhinitis (adjusted OR, 0.51; 95% CI, 0.30 to 0.88). However, other factors, including sputum eosinophils >5%, exhaled no >39 ppb, reversibility of FEV1 >15%, sputum neutrophils >64%, present smoker/ex-smoker, untreated period >10 yr, male, family history of asthma, a college education or advanced degree, and bronchial wall thickening >65%, were not independently associated with the development of FAO in multivariate analysis (**Table 4**).

Airway remodeling evaluated by bronchoscopy and HRCT Scans

Endobronchial biopsy specimens: ASM (a percentage of area in relative to the total biopsy area) and RBM thickness for endobronchial biopsy specimens, WT and WA% for HRCT. The SMA% was significantly greater in the group with PAO than in the group with RAO (15.83%±2.32% [n=9] vs. 8.0%±1.68% [n=7], *P*=0.02; **Figure 1A**). The RBM was significantly thicker in the group with PAO than in the group with RAO (16.27±2.32 μm [n=9] vs. 8.71±2.41 μm [n=7], *P*=0.042; **Figure 1B**). SMA% was correlated with WA% in all subjects, with a trend in the PAO group (r=0.64, *P*=0.04) (**Figure 2A**) but not in the RAO group; RBM was also correlated with WA% in all subjects but not in the RAO group (**Figure 2B**), in the PAO group (r=0.78, P=0.012). No correlation existed in either SMA% or RBM in WT (data not shown).

HRCT: No statistically significant differences between the groups were found (see **Table 3**). There was also no significant correlation between the above HRCT-derived parameters and any of the features of remodeling (data not shown).

Evaluations of cytokines in induced sputum and serum

We found that there were no between-group differences in the serum concentrations of total IgE, MMP9, VEGF, and ADAM33 in the induced sputum and serum. Complete blood cell analysis showed no significant differences in neutrophil and eosinophil counts and there were no differences in induced sputum analysis between groups (**Table 5**).

Discussion

In the present study, we found several lines of evidence for the greater degrees of airway remodeling in the group of patients with severe asthma in Chinese Han population. We observed that PAO in patients with severe asthma was significantly associated with longer dis-

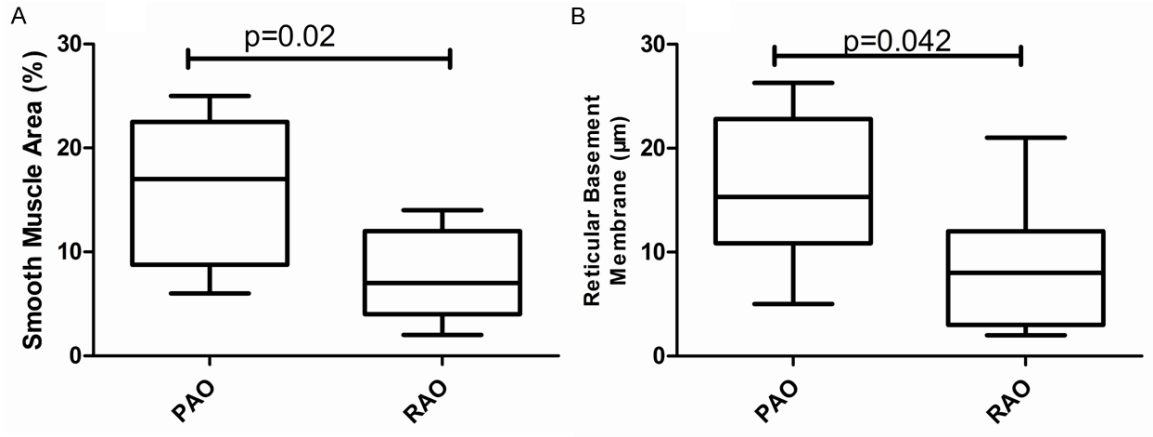

Figure 1. Comparison of smooth muscle area (A) AND reticular basement membrane (RBM) thickness (B) with PAO asthma, RAO asthma. Results are presented as individual data points with median bar.

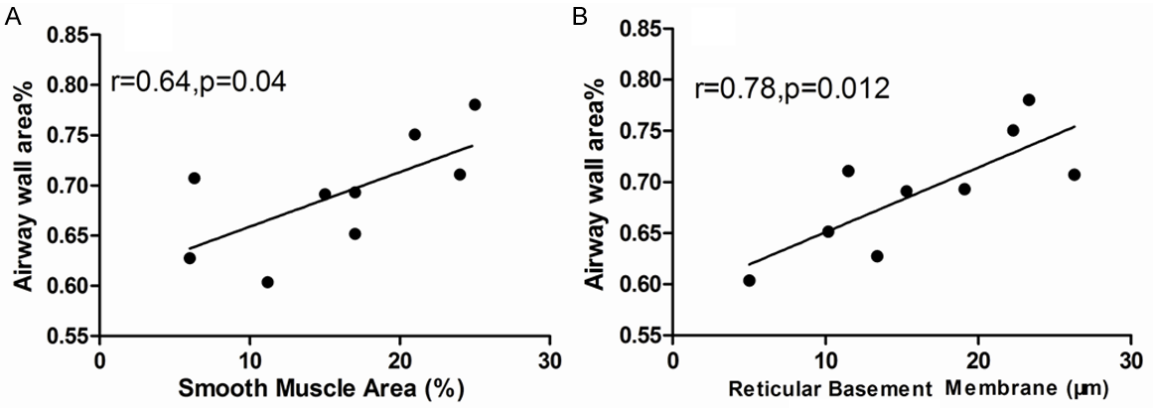

Figure 2. Correlation between WA% (a measure of airway wall thickness on HRCT, see the Methods section for details) and SMA% (A) and RBM thickness (B) for 9 subjects who underwent HRCT and a biopsy in persistent airflow obstruction.

ease duration, less common allergic rhinitis, less common atopy, in the forms of the increased RBM thickness and SMA on endobronchial biopsy specimens, particularly. However, analyses of sputum and serum levels of MMP9, VEGF, and ADAM33, and the biomarkers of inflammation and remodeling, respectively did not distinguish this population from other subjects with severe asthma and nor did the airway thickness parameters on HRCT.

The significant demographic and clinical differences between the PAO and RAO groups suggest the presence of at least two distinct patterns of disease progression in patients with severe asthma. Consistent with that reported in other studies [15, 16], PAO was associated with more severe asthma and longer disease duration. A study analysis also showed that the adults without rhinitis combined with asthma had more severe disease and airway obstruction than patients with rhinitis combined with asthma did [17]. However, we found that the non-atopic was a risk factor for airway remodeling. However, Rasmussen et.al found that the presence of atopy was not a significant risk factor for airway remodeling, despite a trend toward increased atopy in study members with a persistent low ratio [18]. This study indicates that non-atopic asthma without rhinitis may be a prerequisite factor for the development of PAO, because atopic asthma is usually milder, has an earlier onset and is more commonly accompanied by other allergic diseases such as allergic rhinitis [19]. While the mechanism(s) by which allergy averts PAO are unclear, it may be due to a unique protection derived from the effects of airway structure, mechanical force, or inflammatory and related repair [15].

Table 5. Laboratory data in patients with severe asthma

Characteristics	PAO	RAO	*P* value
N	117	125	
WA%	70.59±59.74	70.25±64.08	0.895
WT	0.12±0.23	0.12±0.13	0.854
Sputum neutrophils, %*	71.92±22.40	75.38±23.28	0.557
Sputum eosinophils, %*	2.14±2.16	0.94±1.16	0.011
Atopy. no.	33/102	90/125	0.000
IgE (IU/ml)	219.76±225.97	174.02±233.51	0.558
MMP9 in blood	369.53±95.27	271.89±501.74	0.877
VEGF in blood	49.57±106.34	69.51±130.05	0.371
ADAMM33 in blood	15.76±34.95	15.02±25.90	0.898
MMP9 in sputum	409.21±219.72	387.59±207.10	0.660
VEGF in sputum	53.38±42.19	57.15±43.00	0.691
ADAMM33 in sputum	14.49±11.53	14.19±11.80	0.863

*Successful sputum counts were done in 64 subjects: 35 patients with PAO (n=119), 29 patients with RAO (n=116). 31 subjects underwent HRCT. Data are presented as mean ± SD.

An increase in airway wall area, including smooth muscle, and airway narrowing with an increasing duration of severe asthma were found [4]. Modeling experiments predict that greater muscle thickness allows the development of greater airway wall tension, overcoming the intrinsic impedances to airway narrowing and causing more airway constriction [20]. Our study showed that adequate bronchodilator and anti-inflammatory therapy might be expected to reverse the obstruction (**Table 3**). There has been evidence for persistent inflammation, as reflected by the increased levels in FeNO, but not in sputum differential cell counts, supporting the idea of persistence of stimuli for shortening the duration of ASM.

The association between PAO and the increased ASM and RBM thickness were expected. However, results did not agree with those in the study by Kaminska et. al. [21]. The reason for this disparity can be due to the differences in studied populations, the number of patients, and the inclusion criteria of patients. The noninvasive assessment of airway remodeling remains problematic. We attempted to measure airway thickness on HRCT as a marker of airway remodeling. However, no differences between groups were found, and there was no correlation between wall thickness and FEV1 and others. We examined the sputum and serum levels of MMP-9, VEGF and ADAM33 because they are believed to be involved in airway remodeling. However, we found no significant differences between the groups.

Our study has some limitations. First, the population of patients with severe asthma enrolled in the study might not be the representative of all Chinese patients with severe asthma, although our medical center is the biggest one in Asia. Hence, further studies with larger populations from different representative regions in China are also needed to confirm our findings. Second, as this study was the observational one, the patients may have been diverse in term of the types of medications and adherence to medications. These differences might have affected on the clinical course and the proportion of PAO and, Third, PAO was defined one year after enrollment, this is a relatively short period of following-up.

In conclusion, this study is the first to report the risk factors that may be related to PAO in patients with severe asthma in Chinese Han population. We observed that the patients with severe asthma with PAO had less common atopic, no rhinitis and a longer disease duration and that they had more remodeling of ASM and RBM. ASM remodeling was found to be correlated with airway thickness on HRCT. Asthmatic patients with PAO with increased ASM and RBM might benefit most from the procedure.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chuntao Liu, Department of Respiratory Medicine, West China Hospital, Sichuan University, 37 Guoxuexiang, Chengdu 610041, Sichuan, China. Fax: +86-028-85423872; E-mail: zll280831@163.com

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