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Small airway disease: A different phenotype of early stage COPD associated with biomass smoke exposure

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ABSTRACT

Background and objective: **Chronic exposure to biomass smoke (BS) can significantly compromise pulmonary function and lead to chronic obstructive pulmonary disease (COPD). To determine whether BS exposure induces a unique phenotype of COPD from an early stage, with different physiopathological features compared with COPD associated with smoking (cigarette-smoke (CS) COPD), we assessed the physiopathology of early COPD associated with BS exposure (BS COPD) by incorporating spirometry, high-resolution computed tomography (HRCT) imaging, bronchoscopy and pathological examinations.**

Methods: **In this cross-sectional study, we recruited 29 patients with BS COPD, 31 patients with CS COPD and 22 healthy controls, including 12 BS-exposed subjects who did not smoke and 10 healthy smokers without BS exposure. Spirometry, HRCT scans, bronchoscopy and bronchial mucosa biopsies were performed to assess lung function, emphysema and air trapping, as well as the pathological characteristics and levels of inflammatory cells in bronchoalveolar lavage fluid (BALF).**

Results: **Among COPD patients with mild-to-moderate airflow limitation, BS exposure caused greater small airway dysfunction in BS COPD patients, although these patients had less emphysema and air trapping, as detected by HRCT ($P < 0.05$). We also observed significantly thicker basement membranes and greater endobronchial pigmentation in BS COPD than in CS COPD ($P < 0.05$). Moreover, patients with BS COPD exhibited greater macrophage and lymphocyte infiltration but reduced neutrophil infiltration in their BALF ($P < 0.05$).**

Conclusion: **We used both radiology and pathology to document a distinct COPD phenotype associated with BS exposure. This is characterized by small airway disease.**

SUMMARY AT A GLANCE

This is the first quantitative study of early stage chronic obstructive pulmonary disease (COPD) associated with biomass smoke exposure. Compared with smoking-associated COPD, there are differences in small airway dysfunction, emphysema, air trapping, basement membrane thickness and immune cell influx. This suggests a different phenotype with features of small airway disease.

Key words: chronic obstructive pulmonary disease, pathology, radiology and other imaging, respiratory function tests.

Abbreviations: %GV, percentage of gas volume; 6MWD, 6-min walk distance; 6MWORK, 6-min walk work; BAF, bronchial anthracofibrosis; BALF, bronchoalveolar lavage fluid; BM, basement membrane; BMT, BM thickness; BS, biomass smoke; BSNormal, normal control subjects exposed to only BS; CS, cigarette smoke; CSNormal, normal control subjects exposed to only CS; CT, computed tomography; EI, emphysema index; FEV_1, forced expiratory volume in the first second; FEV_1%pred, predicted percentage of FEV_1; FVC, forced vital capacity; FVC% pred, predicted percentage of FVC; HU, Hounsfield units; LVex/in, expiratory-to-inspiratory ratio of lung volume; MEF25, MEF50 and MEF75, 25%, 50% and 75% of maximum expiratory flow, respectively; MLDex/in, expiratory-to-inspiratory ratio of mean lung density; MMEF, maximum mid-expiratory flow; MMEF% pred, predicted percentage of MMEF; RVC –860 to –950, relative volume change at –860 to –950 HU; VC_{max}%pred, predicted percentage of maximum vital capacity.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality worldwide and represents a substantial economic and social burden.[1] Smoking is a major cause of COPD that has been a focus of research in developed countries; however, biomass smoke (BS) exposure can also lead to COPD,[2] although its impact has generally been neglected by researchers.

A growing body of research indicates that there are significant differences between BS COPD and cigarette-

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smoke (CS) COPD,[3] including the pathogenesis, clinical traits, prognosis and response to treatment of the disease.[4] BS and CS induce different characteristics in patients with COPD, as assessed via CT scanning. In addition, post-mortem analyses of lung morphology have demonstrated that patients with BS COPD have higher levels of lung fibrosis and pigment deposition but less emphysema and epithelial damage.[5] This evidence has led some experts to propose BS COPD as a distinct COPD phenotype[6]; however, further research is required to support this view as some experts consider BS COPD to be a distinct disease, rather than a COPD phenotype.[7] The key point of the argument is whether the physiopathological mechanisms differ between BS COPD and CS COPD. To determine whether BS induces a different phenotype of COPD with different physiopathological features, we assessed the physiopathology of BS COPD via spirometry, high-resolution computed tomography (HRCT) imaging, bronchoscopy and pathological examinations. The data from this study may provide new insights into COPD.

METHODS

Design

This cross-sectional study conducted in Wengyuan, a rural region in southern China, enrolled 60 patients with COPD (29 with BS exposure vs 31 with CS exposure) and 22 healthy controls (12 with BS exposure vs 10 with CS exposure). Spirometry, HRCT scans, bronchoscopy and bronchial mucosa biopsies were performed to assess lung function, emphysema and air trapping, as well as the pathological characteristics and levels of inflammatory cells in bronchoalveolar lavage fluid (BALF). Detailed methods are provided in Appendix S1 (Supplementary Information).

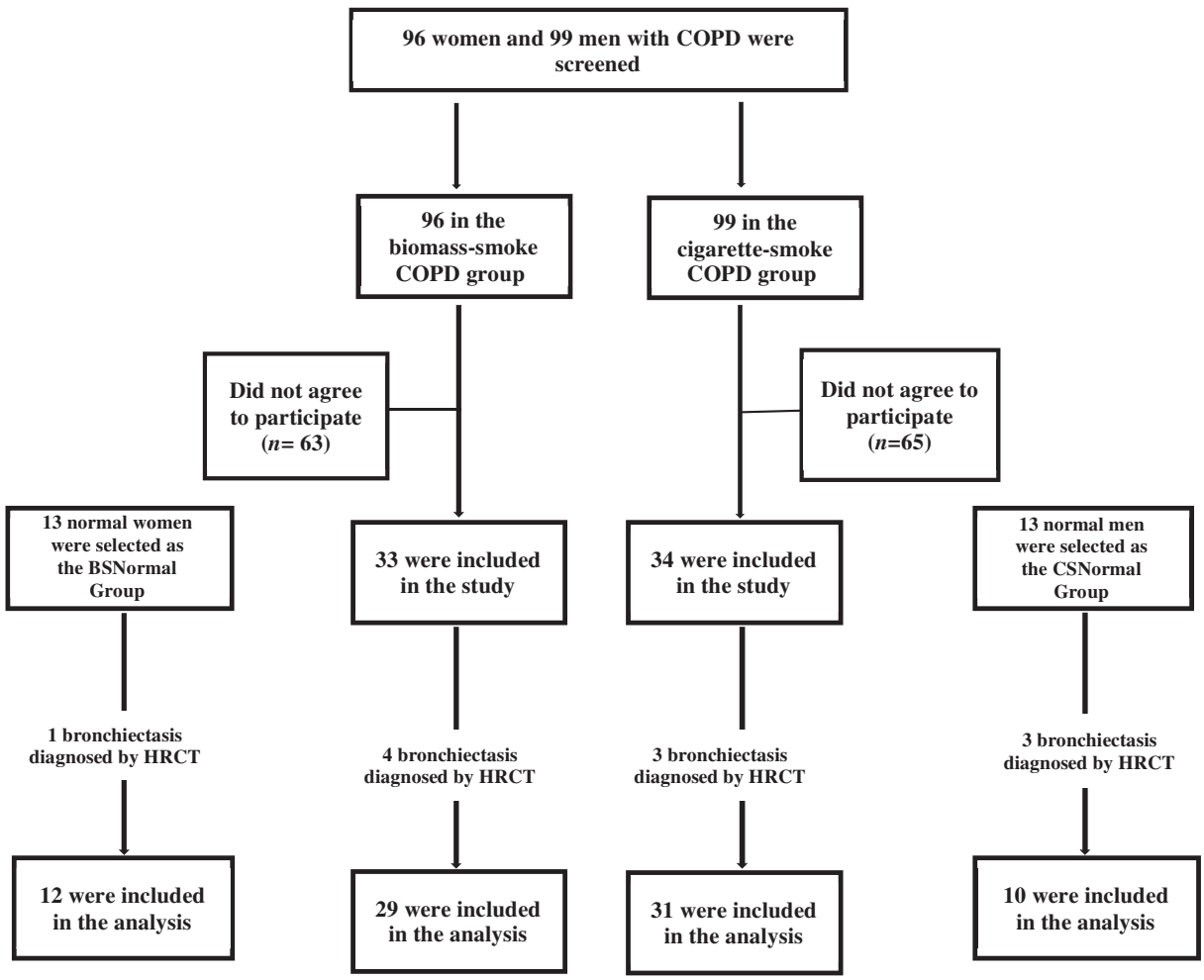

Figure 1 Flow chart of subject recruitment. In total, 96 patients with biomass-smoke (BS) COPD and 99 patients with cigarette-smoke (CS) COPD were screened for eligibility. Thirty-three patients with BS COPD and 34 patients with CS COPD underwent complete HRCT examinations and specimen collection. Based on the HRCT examinations, four patients in the BS COPD group and three patients in the CS COPD group were diagnosed with bronchiectasis and excluded from the study. Thirteen subjects were recruited for both the BSNormal (normal control subjects exposed to only BS) and CSNormal (normal control subjects exposed to only CS) control groups. Based on the HRCT examinations, one subject in the BSNormal group and three subjects in the CSNormal group were diagnosed with bronchiectasis and excluded from the study. Finally, 60 COPD patients and 22 normal subjects were recruited: 29 women in the BS COPD group, 31 men in the CS COPD group, 12 women in the BSNormal group and 10 men in the CSNormal group.

Study participants

The enrolled subjects were classified into four groups according to their exposure: (i) patients with BS COPD, (ii) patients with CS COPD, (iii) normal control subjects exposed to only BS (BSNormal) and (iv) normal control subjects exposed to only CS (CSNormal).

The diagnosis of COPD was made in accordance with the guidelines of the Global Initiative for Chronic Obstructive Lung Disease (GOLD).[8] To reduce the impact of confounding factors, each participant associated with BS was matched by age (within 5 years), place of origin and post-bronchodilator predicted percentage of forced expiratory volume in the first second (FEV_1%pred) (within 10%) to a participant associated with CS. Based on the inclusion and exclusion criteria provided in Appendix S1 (Supplementary Information), 60 COPD patients and 22 normal subjects were recruited: 29 women in the BS COPD group, 31 men in the CS COPD group, 12 women in the BSNormal group and 10 men in the CSNormal group (Fig. 1). The study was approved by the Medical Ethics Review Committee of the Guangzhou Institute of Respiratory Diseases, and all participants provided written informed consent.

Procedures and outcomes

Questionnaires

All subjects completed the questionnaire from the COPD Epidemiological Survey in China,[9] the modified Medical Research Council (mMRC) Questionnaire, the COPD Assessment Test (CAT) and a 6-min walk test. The 6-min walk work (6MWORK) values were calculated as the 6-min walk distance (6MWD) × body weight.[10] Exposure to BS was defined as the use of biofuel (wood, charcoal, grass and crop residues) for cooking or heating ≥1 year.[11] The BIOFUEL-index was defined as the cumulative exposure to BS, which was calculated by multiplying the number of years spent cooking with wood stoves by the average daily number of hours spent in the kitchen.[12]

Spirometry

All subjects underwent pre- and post-bronchodilator spirometry in accordance with the criteria recommended by the American Thoracic Society (ATS) standards. Measurements were performed in triplicate for each subject, and representative data that fulfilled the criteria are reported. Predicted equations were derived using reference values from the European Coal and Steel Community (1993) and applying conversion factors (male: 0.95, female: 0.93).[13]

High-resolution computed tomography

HRCT was performed at suspended full inspiration and expiration (120 kV, 250 mA) using a multidetector row CT scanner (Aquilion 16, Toshiba, Tokyo, Japan). Two radiologists blinded to the exposure history of each subject independently scored each scan.

Quantitative assessments of emphysema were performed using custom software (LungCAD1.1, Neusoft, Shenyang, China). Emphysema was quantified by measuring the emphysema index (EI) of each patient, which was defined as the percentage of voxels less than −950 Hounsfield units (HU) on inspiratory CT scans,[14] and the percentage of gas volume (%GV), which was defined as the ratio of gas volume to lung volume on inspiratory CT scans.

Air trapping was quantified by measuring the relative volume change at −860 to −950 HU (RVC −860 to −950), defined as the percentage change in relative area with attenuation values from −860 to −950 HU between inspiratory and expiratory CT scans.[15] We also calculated the expiratory-to-inspiratory ratio of mean lung density (MLDex/in)[16] and the expiratory-to-inspiratory ratio of lung volume (LVex/in).

Bronchoscopy

All subjects underwent bronchoscopy, BALF was collected with 200 mL of 0.9% NaCl and four to six endobronchial biopsy specimens were taken from the right lower lobes.

Biopsy samples were embedded in paraffin blocks; slices (4 μm thick) were stained with haematoxylin and eosin (HE) and used to evaluate basement membrane (BM) thickness (BMT) and the status of the epithelium. Only sections perpendicular to the BM were selected for measurement. At least 40 measurements at 20-μm intervals were obtained for each subject in accordance with the method developed by Sullivan *et al.*[17]

Statistical analysis

All statistical calculations were performed using SPSS 20.0 (IBM SPSS V.20.0, Armonk, NY, USA). Numeric values are expressed as the means ± SD unless otherwise indicated. The Mann–Whitney U-test and Kruskal–Wallis analysis of variance test were applied to compare two and more than two unrelated samples, respectively. If each of the variables had only two values, a chi-square test with the Yates correction for continuity or Fisher's exact test was used. The Wilcoxon test was used to analyse differences between related variables. Statistical significance was accepted at P-values <0.05.

RESULTS

Baseline characteristics

There were no significant differences in age or weight among the four groups despite the significantly lower height and greater BMI of the BS COPD group compared with the CS COPD group. The BIOFUEL-index for BS COPD and the smoking index for CS COPD were significantly higher than the values of their respective control groups ($P < 0.05$). The expectoration ratio of BS COPD was significantly lower than that of CS COPD ($P < 0.05$). Both the 6MWD and 6MWORK values of BS COPD were significantly lower than those of CS COPD ($P < 0.05$) (Table 1).

Comparison of pulmonary ventilation function

Although no significant difference was observed in FEV_1%pred or FEV_1/forced vial capacity (FVC) between

Table 1 Baseline characteristics

		BSNormal (n = 12)	BS COPD (n = 29)	CS COPD (n = 31)	CSNormal (n = 10)	$P^{\dagger}$
Characteristics						
Sex (men/women)		0/12	0/29	31/0	10/0	NA
Age (years)		59.4 ± 9.0	65.3 ± 8.8	64.9 ± 5.9	64.2 ± 7.1	0.144
BS-exposure years		41.0 ± 14.1	41.6 ± 12.2	0	0	NA
BS-exposure hours		1.7 ± 1.3	2.6 ± 1.2[‡]	0	0	NA
BIOFUEL-index		62.9 ± 41.9	102.4 ± 47.8[‡]	0	0	NA
Smoking index (pack-years)		0	0	53.7 ± 18.2[‡]	40.5 ± 20.1	NA
Height (cm)		150.0 ± 7.8	148.5 ± 5.8[§]	162.2 ± 4.5	160.2 ± 3.7	<0.01
Weight (kg)		49.1 ± 7.0	50.6 ± 11.5	54.5 ± 7.5	57.6 ± 8.6	0.064
BMI		21.8 ± 2.6	23.0 ± 5.1[§]	20.7 ± 2.6	22.9 ± 3.5	0.127
COPD assessment						
Cough[¶]		NA	9 (31.0)	13 (41.9)	NA	NA
Expectoration[¶]		NA	8 (27.6)[§]	17 (54.8)	NA	NA
Wheezing[¶]		NA	6 (20.7)	9(29.0)	NA	NA
Dyspnoea[¶]		NA	6 (20.7)	4 (12.9)	NA	NA
CAT		NA	5.0 ± 4.6	6.2 ± 6.2	NA	NA
mMRC		NA	0	0	NA	NA
6MWD (m)		NA	424.41 ± 72.25[§]	473.1 ± 43.6	NA	NA
6MWORK (kg m)		NA	21518.6 ± 6359.1[§]	25869.6 ± 4777.5	NA	NA
Combined COPD assessment[¶]	A	NA	23 (79.3)	23 (74.2)	NA	NA
	B	NA	6 (20.7)	8 (25.8)	NA	NA

Values were given as mean ± SD unless otherwise indicated.

[†]Comparison among four groups.

[‡]$P < 0.05$ comparison between COPD group and control group.

[§]$P < 0.05$ comparison between COPD groups.

[¶]Values given as numbers of patients (%).

6MWD, 6-min walk distance; 6MWORK, 6-min walk work (6MWD × body weight); BIOFUEL-index, BS exposure hour-years; BS, biomass smoke; BS-exposure hours, the average daily number of hours spent in the kitchen; BS-exposure years, the number of years spent cooking with wood stoves; BSNormal, normal control subjects exposed to only BS; CAT, COPD Assessment Test; combined COPD assessments, assessments of symptoms, classification of severity of airflow limitation and the risk of exacerbations; CS, cigarette smoke; CSNormal, normal control subjects exposed to only CS; mMRC, modified Medical Research Council; NA, not applicable; smoking index, tobacco smoke exposure pack-years.

BS COPD and CS COPD, both the predicted percentage of maximum vital capacity (VC_{max}%pred) and predicted percentage of FVC (FVC%pred) values of BS COPD were significantly higher than those of CS COPD ($P < 0.05$) (Table 2).

Comparison of small airway function

The 25%, 50% and 75% of maximum expiratory flow (MEF25, MEF50 and MEF75, respectively) and maximum mid-expiratory flow (MMEF) values of BS COPD were significantly lower than those of CS COPD and BSNormal ($P < 0.05$). The MEF25, MEF50, MEF75 and MMEF values of CS COPD were significantly lower than those of CSNormal ($P < 0.05$). The predicted percentage of MMEF (MMEF%pred) values of BS COPD and CS COPD were significantly lower than those of their respective control groups ($P < 0.05$) (Table 2).

Features of HRCT

Both the EI and %GV values of BS COPD were significantly lower than those of CS COPD ($P < 0.05$). The EI and %GV values of the two COPD groups were significantly greater than those of the control groups ($P < 0.05$) (Table 2).

The RVC −860 to −950 of BS COPD was significantly lower than that of CS COPD ($P < 0.05$). Both MLDex/in and LVex/in remained unchanged regardless of exposure or disease state (Table 2).

Pathological characteristics

The BMT of BS COPD was significantly greater than that of CS COPD and BSNormal ($P < 0.01$) (Fig. 2, Table 2). We furthered examined a range of tissue abnormalities, including squamous cell metaplasia, goblet cell hyperplasia and epithelial cell shedding. However, we did not observe any significant differences among the four groups (data not shown).

Bronchial anthracofibrosis

Bronchoscopic observations revealed that more patients with BS COPD were affected with bronchial anthracofibrosis (BAF, 30.4%) than those with CS COPD (3.7%). BS exposure also tended to induce more BAF sites, with the majority of sites located in the right

Table 2 Physiopathological features

	BSNormal (n = 12)	BS COPD (n = 29)	CS COPD (n = 31)	CSNormal (n = 10)	$P^{\dagger}$
Pre-bronchodilator pulmonary ventilation function					
VC_{max}%pred (%)	105.2 ± 15.6	112.5 ± 20.9‡	101.6 ± 15.2	99.0 ± 14.3	0.061
FVC%pred (%)	106.9 ± 16.1	114.3 ± 21.5‡	103.6 ± 15.9	101.0 ± 15.4	0.086
FEV_1%pred (%)	105.5 ± 18.7	83.9 ± 18.6§	80.2 ± 15.5§	100.6 ± 14.2	<0.001
FEV_1/FVC (%)	81.5 ± 5.7	60.5 ± 7.9§	61.1 ± 7.7§	79.0 ± 3.4	<0.001
Post-bronchodilator pulmonary ventilation function					
VC_{max}%pred (%)	108.5 ± 14.6	114.4 ± 19.3‡	103.5 ± 14.9	101.3 ± 15.4	0.050
FVC%pred (%)	109.4 ± 16.5	116.6 ± 21.6‡	104.6 ± 15.7	102.6 ± 14.3	0.048
FEV_1%pred (%)	107.0 ± 19.6	87.2 ± 19.1§	81.6 ± 14.6§	102.0 ± 13.4	<0.001
FEV_1/FVC (%)	80.80 ± 5.0	61.6 ± 7.5§	61.7 ± 7.4§	78.8 ± 4.0	<0.001
Pre-bronchodilator small airway function					
MEF25 (L/S)	4.15 ± 0.82	2.54 ± 1.12‡§	3.41 ± 1.23§	5.48 ± 1.21	<0.001
MEF50 (L/S)	2.71 ± 0.89	1.06 ± 0.60‡§	1.49 ± 0.77§	3.33 ± 0.92	<0.001
MEF75 (L/S)	0.68 ± 0.28	0.24 ± 0.17‡§	0.41 ± 0.23§	0.88 ± 0.29	<0.001
MMEF (L/S)	1.80 ± 0.48	0.65 ± 0.32‡§	0.99 ± 0.48§	2.29 ± 0.56	<0.001
MMEF%pred (%)	65.3 ± 19.1	24.2 ± 10.9‡§	31.7 ± 14.4§	73.8 ± 15.4	<0.001
Post-bronchodilator small airway function					
MEF25 (L/S)	4.06 ± 0.91	2.56 ± 1.11‡§	3.42 ± 1.21§	5.51 ± 1.19	<0.001
MEF50 (L/S)	2.87 ± 0.79	1.09 ± 0.60‡§	1.44 ± 0.63§	3.24 ± 0.88	<0.001
MEF75 (L/S)	0.68 ± 0.27	0.25 ± 0.17‡§	0.40 ± 0.23§	0.88 ± 0.27	<0.001
MMEF (L/S)	1.81 ± 0.46	0.7 ± 0.31‡§	1.03 ± 0.47§	2.33 ± 0.56	<0.001
MMEF%pred (%)	65.9 ± 18.5	26.1 ± 10.7§	33.1 ± 13.9§	75.0 ± 15.6	<0.001
Emphysema assessment					
EI (%)	9.7 ± 3.1	17.4 ± 10.1‡§	23.6 ± 5.9§	15.7 ± 2.8	<0.01
%GV (%)	84.1 ± 7.3	88.0 ± 6.7‡§	93.4 ± 1.9§	89.8 ± 2.5	<0.01
Air-trapping assessment					
RVC –860 to –950 (%)	–14.3 ± 22.4	–3.3 ± 18.0‡	12.2 ± 10.2§	–7.6 ± 9.7	<0.01
MLDex/in	0.85 ± 0.06	0.88 ± 0.06	0.87 ± 0.07	0.84 ± 0.08	0.39
LVex/in	0.61 ± 0.14	0.64 ± 0.15	0.66 ± 0.15	0.64 ± 0.17	0.83
Comparison of BMT					
BMT (μm)	3.8 ± 2.7	11.5 ± 3.9‡§	4.4 ± 2.8	3.4 ± 1.9	<0.01
Cytological detection in BALF					
Neutrophil (%)	29.5 ± 27.5	32.5 ± 29.1‡	54.4 ± 29.8§	26.6 ± 27.2	0.01
Macrophage (%)	66.3 ± 26.8	60.6 ± 28.0‡	41.8 ± 31.8§	69.8 ± 27.4	0.02
Eosinophilic (%)	1.6 ± 2.7	1.0 ± 1.9	3.0 ± 6.8	1.3 ± 1.3	0.43
Lymphocyte (%)	2.6 ± 2.3	5.9 ± 7.4‡§	0.7 ± 1.0	2.3 ± 2.7	0.002
BAL recovery (mL)	83.4 ± 5.0	81.9 ± 8.3	79.6 ± 7.7	83.8 ± 8.7	0.31

Values were given as mean ± SD.

†Comparison among four groups.

$^{\ddagger}P < 0.05$ comparison between COPD groups.

$^{\S}P < 0.05$ comparison between COPD group and control group.

%GV, percentage of gas volume; BAL, bronchoalveolar lavage; BALF, BAL fluid; BMT, basement membrane thickness; BS, biomass smoke; BSNormal, normal control subjects exposed to only BS; CS, cigarette smoke; CSNormal, normal control subjects exposed to only CS; EI, emphysema index; FEV_1, forced expiratory volume in the first second; FEV_1%pred, predicted percentage of FEV_1; FVC, forced vital capacity; FVC%pred, predicted percentage of FVC; HU, Hounsfield units; LVex/in, expiratory to inspiratory ratio for lung volume; MEF25, MEF50 and MEF75, 25%, 50% and 75% of maximum expiratory flow, respectively; MLDex/in, expiratory-to-inspiratory ratio for mean lung density; MMEF, maximum mid-expiratory flow; MMEF%pred, predicted percentage of MMEF; RVC–860 to –950, relative volume change at –860 to–950 HU; VC_{max}%pred, predicted percentage of maximum vital capacity.

lung. None of the healthy control subjects had BAF (Fig. 3).

Bronchoalveolar lavage fluid

We found a significantly higher level of macrophages and lymphocytes in BS COPD than in CS COPD ($P < 0.05$). The proportion of neutrophils in BS COPD was significantly lower than that in CS COPD ($P < 0.05$) (Table 2).

DISCUSSION

In this study, we compared early cases of BS-associated COPD with CS-associated COPD. Nonetheless, we identified some important differences: patients with BS COPD had greater lung capacity, worse small airway function, fewer signs of emphysema, less air trapping and distinct pathological features. There was increased BMT and a greater extent of BAF as well as a higher percentage of macrophages and lymphocytes in BALF.

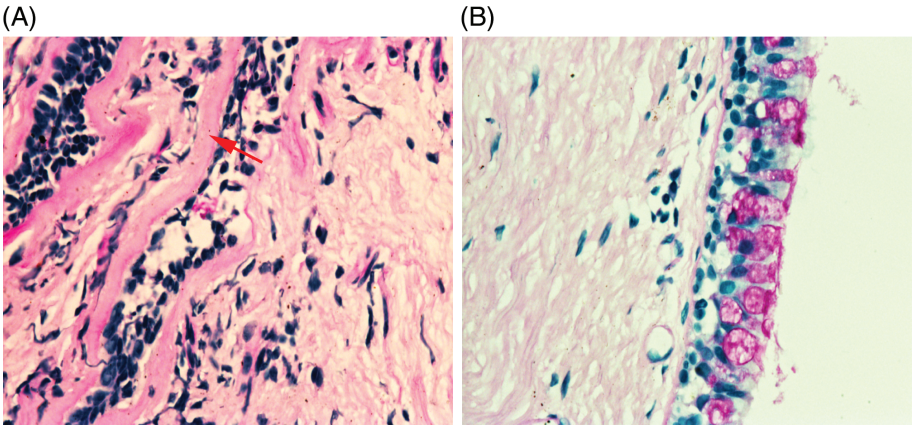

Figure 2 Bronchial biopsy sections from patients with (A) biomass-smoke (BS) COPD and (B) cigarette-smoke (CS) COPD. Compared with CS COPD (B), basement membrane thickness (BMT) of BS COPD (A) was significantly thickened. Red arrow indicates the basement membrane thickening. Original magnification: ×400. Stain: HE. Scale bar = 50 μm.

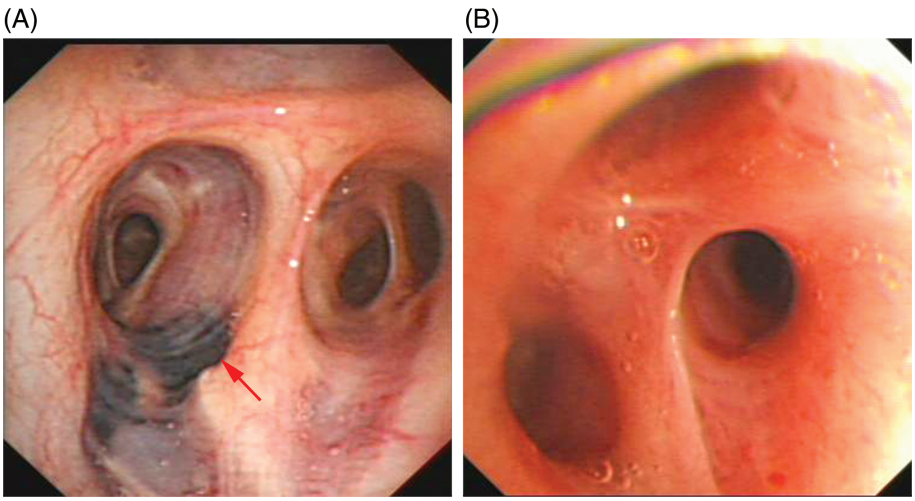

Figure 3 Bronchoscopic observation of patients with (A) biomass-smoke (BS) COPD and (B) cigarette -smoke (CS) COPD. Red arrow indicates bronchial anthracofibrosis (BAF). More patients in the BS COPD (30.4%) group than in the CS COPD (3.7%) group experienced BAF.

As shown by the greater VC_{max}%pred and FVC%pred values and lower MEF25, MEF50, MEF75 and MMEF values in BS COPD compared with CS COPD, patients with BS COPD had greater lung capacity but worse small-airway function.

Emphysema and small airway obstruction are the two major physiopathological processes involved in COPD.[18] Compared with the CS COPD patients, the EI and %GV values of the BS COPD patients in this study were lower, which indicates that milder emphysema is associated with BS COPD. Camp *et al.*[12] found that BS COPD resulted in lower levels of emphysema, which is consistent with our results. This study showed no significant difference in MLDex/in or LVex/in between BS COPD and CS COPD, similar to the results obtained by Camp *et al.*[12] However, the radiologists' ratings in that study showed worse air trapping in BS COPD than in CS COPD,[12] whereas we observed a significantly lower RVC −860 to −950 in BS COPD than in CS COPD, which suggests milder air trapping associated with BS COPD. Air trapping is associated with not only small airway dysfunction but also other physiopathological conditions related to the FEV_1%pred,[19] RV or VC variables.[20,21]

Airway remodelling is the main pathological basis of airway obstruction in COPD patients,[22] and changes in BMT are an important component of airway remodelling, which leads to airway hyperresponsiveness and obstruction.[23] Many studies have demonstrated that asthma patients have increased BMT, similar to the situation observed in COPD.[24] However, the increase in BMT in COPD patients is less severe than that in asthma patients, which make it possible to use the degree of BMT as a diagnostic marker to distinguish COPD from asthma.[25] Our study found that, compared with the CS COPD group, the BM of the patients with BS COPD was significantly thickened. Therefore, we speculate that BS COPD may be different from CS COPD in terms of the mechanisms of airway remodelling.

BAF, which is primarily caused by BS exposure, is commonly found in elderly women in rural areas.[26] Generally, BAF is associated with COPD and affects the right upper and right middle lobes of the lungs.[27] In this study, the frequency of BAF was considerably higher in BS COPD than in CS COPD, and the locations were mainly on the right upper lobe and right middle lobe, consistent with previous studies. Airway inflammation is a major contributory factor to the development of COPD and studies have indicated that neutrophils, macrophages and lymphocytes are all involved.[28–30] Neutrophils are the major cells in the conducting airways, whereas macrophages and lymphocytes are the predominant cells in secretions from

the small airways and parenchyma, which exhibit different patterns of inflammation and different pathologies in COPD.[31] Our study found that the major cells in the BALF of the CS COPD patients were neutrophils, while the major cells in the BALF of the BS COPD patients were macrophages, suggesting that neutrophil-predominant airway inflammation may contribute to the pathogenesis of CS COPD, which mainly exhibits emphysema, while macrophage-predominant airway inflammation may contribute to the pathogenesis of BS COPD, which mainly exhibits small airway dysfunction.

Women in rural southern China seldom use biofuel for heat, except cooking, which led to fewer hours of BS exposure; therefore, the BIOFUEL-index in BS COPD was remarkably low compared with that in some reports.[12,32,33] Poor kitchen ventilation in rural areas of China with poor living conditions leads to very high smoke concentrations,[2] which probably explains the difference between our results and the work of Regalado *et al.*, who found that a mean BIOFUEL-index of 100 hour-years was usually associated with symptoms, rather than FEV_1 deterioration.[32] Additionally, racial differences between Chinese and Mexican women might have caused the discrepant results related to the BIOFUEL-index. Although Camp *et al.* reported that there was no significant difference in BMI between BS COPD and CS COPD,[12] our study showed that BS COPD patients had higher BMI than CS COPD patients, which was consistent with other reports.[34,35] Differences in height, gender and type of exposure may have contributed to this difference.

A limitation to this study is that BS COPD is more common in women, who are more often in charge of cooking, while CS COPD is more common in men, who smoke more often.[3,7] A common problem in studies of BS COPD[12,33] is that subjects with BS COPD are predominantly women, which may lead to sex selection bias. Our study also has this limitation. In traditional Chinese culture, women are predominantly homemakers and consequently experience higher biomass exposure, but they almost never smoke; the opposite is true for men. It is unlikely that we could recruit a woman with COPD only from smoking or a man with COPD only from BS exposure. Therefore, we could only assess male CS COPD patients as a control group. To clarify whether gender contributed to the differences between the subjects with BS and CS exposure, normal subjects with BS or CS exposure were recruited to act as the respective normal control groups. However, we found no significant differences in the analysed parameters between these normal control groups (Table 2), which indicated that gender might not contribute to the differences between the groups with different types of exposure. The age of the BS COPD group tended to be older than that of the BSNormal group, but this difference was not statistically significant. Due to the lower average daily number of hours spent in the kitchen (BS exposure hours), the mean BIOFUEL-index (hour-years) was significantly lower in the BSNormal group than in the BS COPD group, which probably explains the differences between the two groups in the predetermined outcomes, such as FEV_1%pred, FEV_1/FVC and small airway function. Another limitation of our study is that peripheral flow obtained from spirometry (MEF25–75) is not the best tool to assess small airways due to its poor reproducibility and sensitivity.[36,37]

In conclusion, our results indicate that, compared with cigarette smoking, BS exposure induces a different phenotype of COPD that exhibits features of small airway disease from an early stage.

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Supplementary Information

Additional supplementary information can be accessed via the *html* version of this article at the publisher's website.

Appendix S1 Detailed methodology.

