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Application of Inflammatory Markers in Induced Sputum in Stable Chronic Obstructive Pulmonary Disease Patients with Positive Bronchodilation Tests*

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Summary: Positive bronchodilation (BD) tests can be noticed in some stable chronic obstructive pulmonary disease (COPD) patients. The characteristics of airway inflammation in this entity remain unclear. Our study aimed to identify the characteristics of airway inflammation in stable COPD patients with positive BD tests. The airway inflammation was assessed in 88 patients with stable COPD using the examination of induced sputum in the aftermath of lung function and BD tests. Cellular counts and the levels of molecular markers including eosinophil cationic protein (ECP), myeloperoxidase (MPO), interleukin-5 (IL-5), and IL-8 were assayed by Wright's stain, Immuno-CAP system, and ELISA, RT-PCR. Among the 88 patients with stable COPD, 20 (22.7%) showed positive BD tests. The values of eosinophils (4.7%±3.4%) and ECP (90.1±41.6 ng/mL) in induced sputum in stable COPD patients with positive BD tests were markedly elevated as compared with those in stable COPD patients with negative BD tests or in healthy controls (all $P<0.05$), but significantly lower than those in asthmatic patients (all $P<0.01$). The IL-5 in sputum supernatant was significantly decreased in stable COPD patients with positive BD tests as compared with the patients with asthma (12.5±7.8 *vs.* 48.2±26.0 ng/mL; $P<0.01$). However, healthy controls exhibited similar concentrations of IL-5 in induced sputum with patients with stable COPD, whether with positive or negative BD tests (all $P>0.05$). Moreover, the values of neutrophils (61.8%±15.1%), MPO (574.0±111.8 ng/mL), and IL-8 (32.6±13.4 ng/mL) in induced sputum in stable COPD patients with positive BD tests were significantly higher than those in asthmatics or normal controls (all $P<0.01$). However, the values of the above inflammatory markers in induced sputum were similar among stable COPD patients with positive or negative BD tests (all $P>0.05$). The stable COPD patients with positive BD tests may present not only eosinophilic airway inflammation but also neutrophilic airway inflammation.
Key words: airway inflammation; bronchodilation tests; chronic obstructive pulmonary disease; induced sputum

Chronic obstructive pulmonary disease (COPD) is a prevalent, debilitating disease of the lungs with a strong personal impact and economic burden[1]. Typically, this disorder is characterized by progressive and irreversible airflow limitation, which is validated by spirometry with a forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC) ratio being less than 70% after inhalation of bronchodilators[1]. However, COPD is not a simple homogenous disorder physiologically defined only as irreversible airflow limitation, since some patients with COPD may display reversible airflow limitation and airway hyperresponsiveness[2, 3]. It has been reported that a positive bronchodilation (BD) test, which is representative in asthma, could be observed in some stable COPD patients[2]. As is known to all, airway inflammation is of vital importance to the pathogenesis of the common airway conditions, and different types of airway inflammatory responses are indicative of different respiratory diseases[4, 5]. However, the underlying inflammatory pattern in stable COPD patients with positive BD tests is unclear.

Induced sputum examination has been proposed as a valid, safe, and relatively noninvasive method of characterizing airway inflammatory phenotypes in a variety of airway disorders including COPD[6]. Cellular counts and the levels of molecular markers such as eosinophil cationic protein (ECP), myeloperoxidase

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(MPO), or cytokines in sputum supernatant were detected in the examination[6]. Abnormalities in sputum examination usually emphasize the heterogeneity of airway inflammation[6]. It is well recognized that different types of airway inflammatory profiles may have implications as to the corresponding causes as well as to the effects of treatment[6, 7]. A eosinophilic airway inflammation, characterized by an excess proportion of eosinophils and elevated levels of eosinophil-derived mediators like ECP, is typical of asthma and predicts a favorable response to corticosteroids[8]. In contrast, a neutrophilic inflammatory response, associated with an increase in neutrophils and neutrophil proteins such as MPO, can occur in a majority of patients with COPD and predicts a poor response to corticosteroids[5]. Analysis of composition of induced sputum may help to accurately discriminate eosinophilic airway inflammation from noneosinophilic inflammation, and may therefore guide clinical management[7].

Our study aimed to evaluate the frequency of positive BD tests in stable COPD patients, and further to assess the characteristics of airway inflammation in this entity using induced sputum examination.

1 MATERIALS AND METHODS

1.1 Patients and Tissue Specimens

All patients aged 18–75 years with stable COPD attending our respiratory outpatient clinic who fulfilled the entry criteria were included. At entry into the study, COPD was confirmed objectively by the demonstration of chronic airflow limitation: a post-bronchodilator FEV1%FVC ratio of less than 70%, and data regarding severity of COPD were obtained, according to the Gold Initiative for Obstructive Lung Disease (GOLD) criterion[1]. All patients had negative skin-prick test results to standard aeroallergens. None of the patients had reported any acute exacerbation, defined as admission to hospital related to worsening of respiratory symptoms despite treatment or use of antibiotics, steroids, or both captured from a diary of exacerbations within 4 weeks of entry to the study; had actively smoked for at least 6 months before the study entry; or had confounding pulmonary comorbidity or other serious diseases. Patients who had received regular oral corticosteroids were ineligible. In patients taking inhaled corticosteroids, these drugs were discontinued for at least 1 month prior to the study entry.

Thirty asthmatic patients who fulfilled the diagnostic criteria of the Global Initiative for Asthma (GINA)[9] and 30 healthy individuals were recruited and served as controls. All patients who were diagnosed as asthmatics were under uncontrolled stage and did not take any anti-asthma medication before the study entry. Patients with asthma were nonsmokers or ex-smokers for more than 6 months with a pack years of smoking of less than 10 years, had one or more positive skin prick test reactions. The severity of asthma exacerbation was classified as mild, moderate, or severe, using the GINA guidelines[9]. Normal control subjects were asymptomatic, healthy subjects with normal spirometric values (FEV_1 >80% predicted and ratio of FEV_1 to FVC >80%). All subjects had no clinical or radiological evidence of bronchiectasis or pulmonary interstitial fibrosis, and no symptoms suggestive of acute lower respiratory tract infection or oral candidiasis within 8 weeks of entering the study. The study flow-chart is shown in fig. 1. Subjects attended our department on two consecutive days. On the first day of attendance, demographic data were obtained, and allergen skin-prick test, 6-min walking distance (6MWD), modified Medical Research Council (mMRC) scale, and body mass index (BMI), airflow obstruction, dyspnea, and exercise capacity (BODE) index were evaluated. On the second day of attendance, the airway inflammation was assessed using induced sputum examination in the aftermath of lung function and BD tests. Selected sputum portions were assayed for cellular counts and the levels of ECP, MPO, IL-5, and IL-8 using the method of Wright's stain, Immuno-CAP system, and ELISA, RT-PCR respectively.

1.2 Clinical Assessment

At entry anthropometric details of age, gender, BMI, and atopic and childhood respiratory history were collected. Details of smoking status, pharmacologic treatments, and concurrent medical conditions and medications were subsequently recorded. Subjects were also assessed for the presence of a respiratory tract infection and oral candidiasis, and were excluded if either was present. Allergen skin sensitivity was measured by skin-prick test reactions to common allergen extracts, including *Dermatophagoides pteronyssinus*, cat fur, grass pollen, and *Aspergillus fumigatus* solutions. Exercise capacity was evaluated by the 6MWD test, and dyspnea by the mMRC scale. The percentage of BMI, obstructive ventilatory impairment (FEV_1% predicted), mMRC dyspnea scale, and exercise capacity (6MWD values) were integrated into the BODE index.

1.3 Pulmonary Function Tests

Pulmonary function tests (PFTs) were performed according to the standard operating procedure[10], and measured with a Vmax229 Pulmonary Function Instrument (Sensor-Medics, USA). FEV_1 and FVC were measured with the Vmax229 (Sensor-Medics). PFTs results were expressed as percentages of predicted normal values. The best of three consecutive spirometry recordings was used, following the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines[10]. The severity rankings of COPD were based on FEV_1 (% predicted) in accordance with

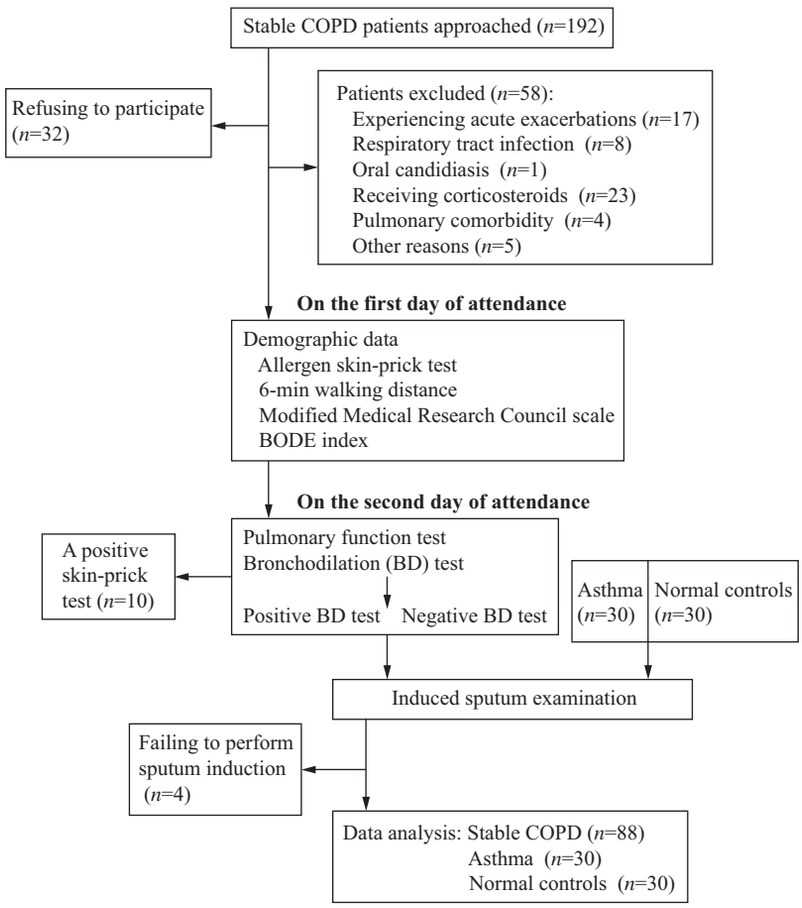

Fig. 1 Study flow-chart

the GOLD guidelines[1]. Patients were subsequently asked to inhale 200 μg salbutamol after baseline evaluation, then PDTs were repeated with a 15-min interval. The positive BD tests, which were defined as a post-bronchodilator increase in FEV_1 >12% and 200 mL from baseline after salbutamol inhalation, were obtained.

1.4 Sputum Induction and Counting

A hypertonic saline solution bronchial provocation challenge and sputum induction were performed as previously described[11]. A differential count of sputum cells was performed using Wright's stain. Five hundred non-squamous cells were counted in Wright's-stained slides and the results were expressed as percentage of the non-squamous counts. A skilled observer who was unaware of the clinical characteristics of the subjects conducted the cellular counts. A cutoff value of 3% is chosen since it is over two standard deviations (SDs) outside the normal range[12].

1.5 Quantification of Molecular Markers in Induced Sputum

The levels of IL-5, IL-8 and MPO were determined by ELISA (FANKE, China). The levels of ECP were measured by the Immuno-CAP system (Pharmacia Diagnostics AB, Sweden). Measurements of the above soluble markers in sputum were done blind to clinical characteristics of the subjects. If sputum induction was not performed on the second visit, the patient was excluded from the final analysis.

1.6 Gene Expression in Human Red Blood Cell Granulocytes of IL-5/IL-8 by Real-time PCR

Total RNA was harvested using QIA shredder (Qiagen, USA) and RNAeasy Mini kit (Qiagen). Real-time PCR was done as described previously. cDNAs were generated from total RNA. Amplifications of 0.1 μg cDNA were carried out using SYBR Green I–based real-time PCR on the MJ Research DNA Engine Opticon Continuous Fluorescence Detection System (MJ Research, Inc., USA). All PCR mixtures contained PCR buffer [final concentration of 10 mmol/L Tris-HCl (pH 9.0), 50 mmol/L KCl, 2 mmol/L $MgCl_2$, and 0.1% Triton X-100], 250 μmol/L deoxynucleotide triphosphate (Roche, USA), 0.5 μmol/L of each PCR primer, 0.5 μL SYBR Green I (Molecular Probes, USA), 5% DMSO, and 1 U Taq DNA polymerase (Promega, USA) with 2 μL cDNA in a 25 μL final volume reaction mix. The samples were loaded into wells of low-profile, 96-well microplates. After an initial denaturation step

of 60 s at 95°C, conditions for cycling were 30 cycles of 30 s at 94°C, 30 s at 52°C, and 1 min at 72°C. Then, the fluorescence signal was measured right after incubation for 5 s at 75°C that followed the extension step, which eliminated possible primer dimer detection. At the end of the PCR cycles, a melting curve was generated to identify the specificity of the PCR product. For each run, serial dilutions of human glyceraldehyde-3-phosphate dehydrogenase (h-GAPDH) were used as standards for quantitative measurement of the amount of amplified DNA. Also, for normalization of each sample, hGAPDH primers were used to measure the amount of hGAPDH cDNA. All samples were run in triplicates and the data were presented as gene-to-GAPDH ratio. The primer sequences were as follows: for IL-5 (forward: 5′-GGATGCCCAGAATAGATG-3′; reverse: 3’-AGGAGGCCAAG ATGAAGTG-5’); for IL-8 (forward: 5′-GGCTTTCAACAAAAGAGTG-3′; reverse: 3′-GTAAGCAAGC CTAATTACCTCT-5′); for GADPH (forward: 5′-CCTGTACGCC AACACAGT-GC-3′; reverse: 5′-ATACTCCTGCTTGCTGATCC-3′). The method was used to analyze relative changes in gene expression.

1.7 Statistical Analysis

All continuous variables were expressed as mean±standard deviations (SD) or median (interquartile range, IQR) and were compared by *t*-tests, Wilcoxon-Mann-Whitney tests, respectively. Categorical variables were expressed as absolute number and proportions. A *P*-value less than 0.05 was considered to represent a statistically significant difference. All statistical analyses were performed using SPSS version 20.0 software (SPSS, USA).

2 RESULTS

2.1 Clinical Characteristics of Subjects

192 well-characterized patients with stable COPD were screened. 104 patients were excluded for the following reasons: experiencing acute exacerbations (*n*=17), respiratory tract infection (*n*=8) or oral candidiasis (*n*=1) during the preceding 4 weeks; receiving oral or inhaled corticosteroids within 1 month of entry to the study (*n*=23); positive skin-prick test (*n*=10); inability to perform sputum induction (*n*=4); having pulmonary comorbidity like bronchiectasis or interstitial lung disease (*n*=4); refusing to participate (*n*=32); other reasons (*n*=5). Eighty-eight patients met our inclusion criteria, and completed our study according to the protocol.

The baseline characteristics of all subjects are shown in table 1. Amongst the 148 subjects, patients with stable COPD tended to be older (65.5±14.0 years) and had a male predominance (*n*=60, 68.2%). The values of BMI were similar in healthy individuals, patients with stable COPD or patients with asthma (all $P>0.05$). A subtotal of 65 (73.9%) of the stable COPD patients, 9 (30.0%) of the asthmatic patients, and 15 (37.5%) of the healthy controls were ex-smokers. And the pack years of tobacco use was 40.0±18.5 years in 65 of the smokers with stable COPD. The distances of 6MWD in stable COPD patients (338.8±97.5 m) were markedly decreased as compared with normal controls (584.3±105.5 m) ($P<0.01$). The severity of all subjects with stable COPD was evaluated on the basis of BODE index, and the results are summarized in table 1. Ten (33.3%) patients with asthma suffered mild

Table 1 Patient characteristics for the individual groups[#]

Parameters	Healthy controls (*n*=30)	Stable COPD (*n*=88)	Asthma (*n*=30)
Age, years	38.6±12.4	65.5±14.0*†	41.5±10.3
Male gender, *n* (%)	22 (55.0%)	60 (68.2%)*†	21 (52.5%)
Body mass index, kg/m^2	27.0±12.4	26.3±12.0	27.7±13.2
Ex-smokers, *n* (%)	15 (37.5%)	65 (73.9%)*†	9 (30.0%)
Pack years of smoking	24.0±12.7	40.0±18.5*†	25.6±15.0
Positive skin-prick test, *n*	0	0	40
6-min walking distance, m	584.3±105.5	338.8±97.5*	–
mMRC scale, *n* (%)	–		–
Grade 0/Grade 1/Grade 2/Grade 3/ Grade 4		14/37/25/8/4 (15.9%/42.0%/28.4%/9.1%/4.5%)	
BODE quartile, *n* (%)	–		–
0–2 (mild)/3–4 (moderate)/ 5–6 (severe)/7–10 (very severe)		21/54/13/0 (23.9%/61.4%/14.8%/0)	
Asthma exacerbation, *n* (%)	–	–	
Mild/Moderate/Severe/Very severe			10/13/6/1(33.3%/43.3%/20.0%/3.3%)
COPD grade, *n* (%)	–		–
1/2/3/4		15/56/17/0 (17.0%/63.6%/19.3%/0)	
FEV_1 (% predicted)	96.2±5.2	60.8±12.8*	62.0±13.2*
FEV_1/FVC (%)	98.3±5.8	58.2±13.4*	63.3±14.0*

[#]Data are represented as mean±standard deviation or number (%). *$P<0.01$ *vs.* healthy controls, †$P<0.01$ *vs.* asthma patients. mMRC: modified Medical Research Council; BODE: body mass index, airflow obstruction, dyspnea, and exercise capacity; COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in the first second of expiration; FVC: forced vital capacity

exacerbation, 13 (43.3%) had moderate exacerbation, 6 (20.0%) had severe exacerbation, and 1 (3.3%) had very severe exacerbation.

2.2 Pulmonary Function and BD Tests

The spirometry results are shown in table 1. Among the 88 patients with stable COPD, 20 (22.7%) had positive BD tests. The mean values of the FEV1% predicted and FEV_1/FVC(%) in patients with stable COPD were significantly reduced as compared with those in normal controls (all $P<0.01$), but were comparable to those of patients with asthma (all $P>0.05$). Concerning the GOLD classification of the severity of the airflow limitation in COPD, there were 15 (17.0%), 56 (63.6%), and 17 (19.3%) subjects, respectively, at grades 1, 2, and 3 in all subjects with stable COPD. None of them corresponded to GOLD 4.

2.3 Cellular Differential Counts and Levels of ECP, MPO in Induced Sputum

The results of the induced sputum examination are shown in table 2. To clarify the inflammatory phenotype of stable COPD patients with positive BD tests, we measured cellular differential counts and the levels of specific inflammatory markers including ECP and MPO in induced sputum. The proportion of eosinophils (4.7%±3.4%) and levels of ECP (90.1±41.6 ng/mL) in induced sputum in stable COPD patients with positive BD tests were markedly elevated as compared with those in stable COPD patients with negative BD tests (all $P<0.01$) or in healthy subjects (all $P<0.01$), but significantly lower than those in asthmatic patients (all $P<0.01$). In addition, the stable COPD patients with positive BD tests had significantly increased proportion of neutrophils (61.8%±15.1%) and concentrations of MPO (574.0±111.8 ng/mL) in induced sputum as compared with those with asthma or healthy subjects (all $P<0.01$). However, the values of neutrophils and MPO in induced sputum had similar performance between stable COPD patients with positive or negative BD tests (all $P>0.05$).

A cutoff value of 3% is indicative of sputum eosinophilia. Seven (35.0%) of the 20 stable COPD patients with positive BD tests displayed a proportion of eosinophils of less than 3%, as shown in fig. 2A. Meanwhile, sputum eosinophilia was observed in 11 (16.2%) stable COPD patients with negative BD tests. For stable COPD patients with positive BD tests, the percentage of sputum eosinophils was marginally higher for grade 2 (5.9%±4.2%) than for grade 1 (3.8%±1.7%) and 3 (3.5%±2.4%). However, it was not statistically increased with COPD severity ($P>0.05$; fig. 2B). In addition, the levels of sputum neutrophils exhibited similar performance among subgroups of different lung functions, as shown in fig. 2D (all $P>0.05$).

2.4 Levels of IL-5 and IL-8 in Induced Sputum Supernatant

The IL-5 levels in induced sputum of the stable COPD patients with positive BD tests were markedly decreased as compared with those in asthma patients (12.5±7.8 *vs.* 48.2±26.0 ng/mL; $P<0.01$). However, the IL-5 levels in induced sputum were not statistically different among healthy subjects, stable COPD patients with positive or negative BD tests ($P>0.05$). The IL-8 levels in sputum supernatant of stable COPD patients with positive BD tests (32.6±13.4 ng/mL) were significantly increased as compared with those in asthmatics or healthy subjects (all $P<0.01$), but there was no significant difference in the IL-8 levels in induced sputum between stable COPD patients with positive or negative BD tests ($P>0.05$).

2.5 Expression Levels of IL-5 and IL-8 mRNA in Stable COPD Patients and Asthmatic Patients

The mRNA expression levels of IL-5 and IL-8 were detected by Real-time PCR in 16 samples of stable COPD patients and asthmatic patients for granulocytes in venous blood. The mRNA expression of IL-5 in the

Table 2 Induced-sputum characteristics[#]

Parameters	Healthy controls (n=30)	Stable COPD (n=88)		Asthma (n=30)
		Positive BD tests (n_1=20)	Negative BD tests (n_2=68)	
Total cell count, 10^6/mL	2.5±0.9	9.0±2.2*‡	8.5±1.8*‡	3.2±1.3*
Viability (%)	70.5±15.2	69.1±18.0	67.2±16.6	68.9±15.7
Cellular differential (%)				
Eosinophils	1.2±1.0	4.7±3.4*†‡	1.9±2.0‡	14.8±8.5*
Neutrophils	29.6±8.8	61.8±15.1*‡	64.0±15.5*‡	46.8±14.5*
Macrophages	62.8±13.8	27.8±8.5*	28.5±9.2*	23.9±9.8*
Lymphocytes	1.8±0.7	3.2±1.2*‡	3.3±1.0*‡	8.0±2.6*
Epithelial cells	3.2±1.2	3.3±1.4	2.9±1.2	3.0±1.5
Molecular markers, ng/mL				
ECP	54.5±18.4	90.1±41.6*†‡	68.3±29.5‡	354.1±188.6*
Myeloperoxidase	65.8±18.7	574.0±118.8*‡	580.2±130.3*‡	208.5±88.3*
Interleukin-5	10.1±4.8	12.5±7.8‡	12.0±5.7‡	48.2±26.0*
Interleukin-8	10.8±5.0	32.6±13.4*‡	30.5±15.1*‡	12.3±5.1

[#]Data are represented as mean±standard deviation. *$P<0.01$ *vs.* healthy controls, †$P<0.01$ *vs.* stable COPD patients with negative BD tests, ‡$P<0.01$ *vs.* patients with asthma. COPD: chronic obstructive pulmonary disease; BD: bronchodilation; ECP: eosinophil cationic protein

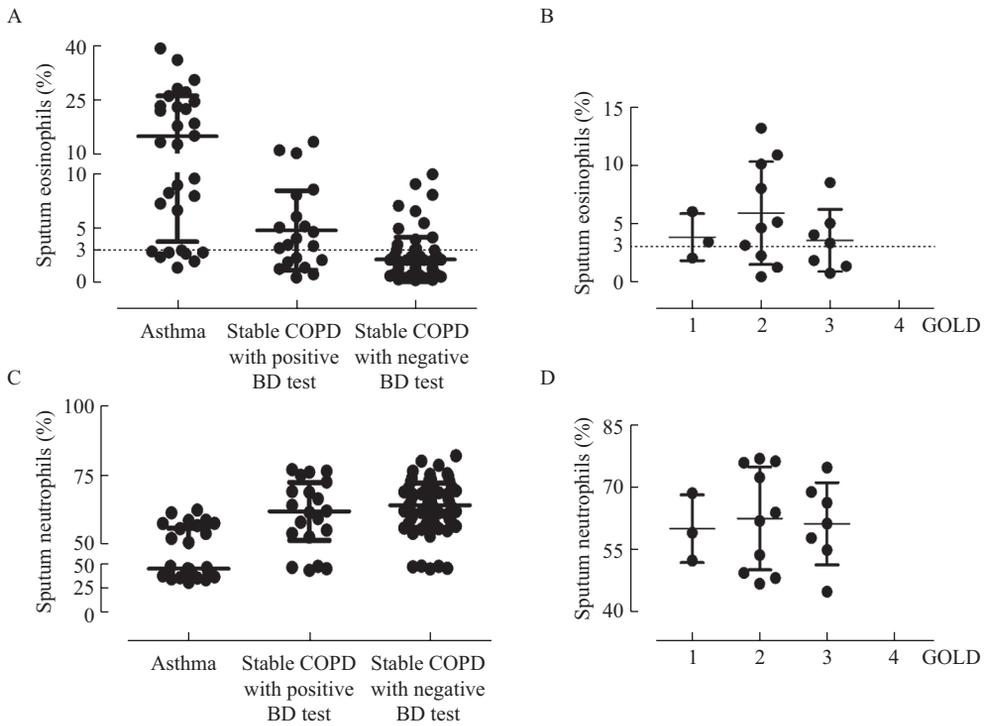

Fig. 2 The levels of sputum eosinophils in individual groups (A) and in stable COPD patients with positive bronchodilation tests (B), the levels of sputum neutrophils in individual groups (C) and in stable COPD patients with positive bronchodilation tests (D)

stable COPD patients was significantly lower than in the asthmatic patients (16.49±2.11 *vs.* 27.83±3.14, $P<0.05$), and that of IL-8 in the stable COPD patients was significantly higher than in the asthmatic patients (10.74±0.93 *vs.* 6.48±0.85, $P<0.05$).

3 DISCUSSION

COPD is a heterogeneous disease associated with significant morbidity and mortality. Current diagnostic criteria based on the presence of fixed airflow obstruction and symptoms do not integrate the complex pathological changes occurring within the lung and they do not define different airway inflammatory patterns. In the current study, 20 (22.7%) of the 88 stable COPD patients had positive BD tests. The stable COPD patients with positive BD tests exhibited significantly elevated values of eosinophils and ECP in induced sputum as compared with stable COPD patients with negative BD tests. Moreover, the values of neutrophilic inflammatory markers including neutrophils and MPO in stable COPD patients with positive BD tests were markedly higher than those in asthmatics or healthy subjects. Consequently, our data demonstrated that stable COPD patients with positive BD tests may present not only eosinophilic airway inflammation but also neutrophilic airway inflammation.

COPD is a complex respiratory disease characterized by incompletely reversible airflow limitation that often develops after the fourth decade of life[1]. International guidelines distinguish between reversible and nonreversible airflow limitation by demonstrating that reversible airflow limitation results in an increase in FEV_1 of >12% and 200 mL after 200 μg of inhaled salbutamol[1]. It is acknowledged that the degree of reversibility of airflow limitation in response to bronchodilators, although limited, varies greatly among individuals with COPD[13]. It was reported that a significant reversibility of airflow limitation may be present in up to 30% of patients with stable COPD[14]. In our present study, 22.7% of the stable COPD patients had an excellent reversibility of airflow limitation after the use of salbutamol, which suggested a positive BD test. According to the GOLD statement and GINA statement, asthma-COPD overlap (ACO) is characterized by persistent airflow limitation with overlapping features of asthma and COPD[15]. Importantly, the descriptive term ACO does not describe a single phenotype of a spectrum of chronic obstructive airway diseases, since COPD and asthma are heterogeneous disorders. The clinical phenotypes and underlying mechanisms of ACO remain controversial. Our data showed that a subgroup of 20 stable COPD patients had positive BD tests, feature traditionally attributing to asthma, however, the characteristics of the airway inflammation phenotype in this entity is less clear.

Assessment of airway inflammation is commonly

recognized as an important aspect in the diagnosis and clinical control between COPD and asthma[4]. COPD and asthma are considered as chronic airway inflammatory diseases, which are typically associated with Th1-mediated immune response in COPD and related to the characteristics of Th2-mediated immunity in asthma[4, 5]. In addition, induced sputum examination is a valid and relatively noninvasive method of characterizing airway inflammatory phenotypes in airway disorders[6]. In our current study, results of cellular differential counts and inflammatory markers in induced sputum were compared among stable COPD patients with positive or negative BD tests, asthmatics, and healthy controls. Our findings demonstrated that stable COPD patients with positive BD tests exhibited markedly elevated levels of eosinophils and ECP in induced sputum when compared with those with negative BD tests. However, the values of eosinophils and ECP in induced sputum in the former population were significantly lower than those of patients with asthma. Therefore, the stable COPD patients with positive BD tests were associated with the evidence, albeit weaker than asthma, of eosinophilic airway inflammation. Meanwhile, our data also revealed that stable COPD patients with positive BD tests had significantly increased amounts of neutrophils and MPO in induced sputum as compared with the subjects with asthma, and that the values of neutrophils and MPO in induced sputum had similar performance between stable COPD patients with positive or negative BD tests. Consequently, the stable COPD patients with positive BD tests were characterized by neutrophilic and eosinophilic airway inflammation. The precise mechanisms behind the presence of eosinophilic airway inflammation in the stable COPD patients with positive BD tests are still debated.

IL-5 is a cytokine produced by the Th2 lymphocytes usually associated with asthmatic airway inflammation, and IL-8, one of the most potent neutrophil chemoattractants in COPD, is mainly produced by macrophages[4, 16]. To further explore the molecular mechanisms possibly contributing to the airway inflammatory phenotype in stable COPD patients with positive BD tests, IL-5 and IL-8 in sputum supernatant were detected. Our data suggested that the stable COPD patients with positive BD tests had markedly decreased levels of IL-5 in induced sputum when compared with asthmatics, however, the former had a significantly increased amounts of IL-8 in sputum supernatant than the latter. These results indicated that the increased values of IL-8 in sputum may be closely related to the recruitment of neutrophils to airways, whereas levels of IL-5 in sputum supernatant may be less relevant to the presence of eosinophilic airway inflammation in this population. It was thus reasonable for us to speculate that the specific mechanisms underlying the pattern of eosinophilic airway inflammation in the stable COPD patients with positive BD tests are far different from those in asthma. The respiratory epithelium is the first line of defense against environmental insults, in particular pathogens, inspired noxious particles such as cigarette fume, and allergens[17]. It is widely accepted that chronic exposure to tobacco smoke leads to increased permeability of the respiratory epithelium, impaired mucociliary clearance, and enhanced release of proinflammatory cytokines and chemokines with consecutive recruitment of macrophages and neutrophils[18, 19]. As a result, the disabled physical barrier may not effectively impede the penetration of allergens, which may eventually lead to the occurrence of allergic airway inflammation.

The weight of evidence indicates that measurement of airway eosinophilia is a reliable predictive index of responsiveness to inhaled and oral corticosteroid therapies in patients with asthma and COPD[13, 20–23]. In sputum, a eosinophil threshold concentration of ≥3% has been recommended for defining eosinophilia in COPD[22, 23]. Interestingly, in patients with COPD who have a significant response to bronchodilators, there is a functional and clinical response to inhaled corticosteroids, which is similar to that observed in patients with asthma[24]. We thus further explored the levels of sputum eosinophils of patients with stable COPD who had a positive BD test. Our results revealed that 7 (35.0%) stable COPD patients with positive BD tests displayed a proportion of eosinophils of less than 3%, and the levels of sputum eosinophils were not correlated with COPD severity. Besides, sputum eosinophilia was observed in 16.2% stable COPD patients with negative BD tests. As a consequence, compared with a positive BD tests, increased eosinophil counts in the sputum may serve as a more reliable index for identifying COPD patients who could respond to corticosteroids treatment. Further studies concerning this issue are needed to better inform treatment decisions for COPD patient.

In conclusion, we have identified a subgroup of stable COPD patients with positive BD tests that are associated with sputum eosinophilia. The presence of a positive BD test in stable COPD is associated with a significant increase in airway inflammatory markers indicative of mixed eosinophilic and neutrophilic airway inflammation.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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