High Mobility Group Protein B1 (HMGB1) in Asthma: Comparison of Patients with Chronic Obstructive Pulmonary Disease and Healthy Controls

Changchun Hou,[1,2]* Haijin Zhao,[1]* Laiyu Liu,[1] Wenjun Li,[3] Xiaoting Zhou,[1] Yanhua Lv,[1] Xiangbo Shen,[1] Zhenyu Liang,[1] Shaoxi Cai,[1] and Fei Zou[3]

[1]Department of Respiration, Chronic Airways Diseases Laboratory, Nanfang Hospital, Southern Medical University, Guangzhou, China; [2]†Department of Internal Medicine, the first affiliated hospital of Guangxi Medical University, Nanning, China; and the [3]School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, China

High mobility group protein B1 (HMGB1) has been implicated as an important mediator in the pathogenesis of asthma and chronic obstructive pulmonary disease (COPD). However, the expression of HMGB1 in plasma and sputum of patients with asthma and COPD across disease severity needs to be defined. The objective of the study was to examine the induced sputum and plasma concentrations of HMGB1 in COPD and asthmatic patients to determine differences in HMGB1 levels between these diseases and their relationship with airway obstruction and inflammatory patterns. A total of 147 participants were enrolled in this study. The participants included 34 control subjects, 61 patients with persistent asthma (according to the Global Initiative for Asthma (GINA) guidelines) and 47 patients with stable COPD (stratified by Global Initiative for Chronic Obstructive Lung Disease (GOLD) status). Spirometry was performed before sputum induction. HMGB1 levels in induced sputum and plasma were determined by enzyme-linked immunosorbent assay. Sputum and plasma concentrations of HMGB1 in patients with asthma and COPD were significantly higher than concentrations in control subjects and were significantly negatively correlated with forced expiratory volume in 1 s (FEV_1), FEV_1 (% predicted) in all 147 participants. The levels of HMGB1 in induced sputum of COPD patients were significantly higher than those of asthma patients and healthy controls ($P < 0.001$). This difference was present even after adjusting for sex, age, smoking status, daily dose of inhaled corticosteroids and disease severity. There were no significant differences in HMGB1 levels between patients with eosinophilic and noneosinophilic asthma. HMGB1 levels in asthmatic and COPD patients were positively correlated with neutrophil counts and percentage of neutrophils. In multivariate analysis, the two diseases (asthma and COPD) and disease severity were independent predictors of sputum HMGB1, but not smoking, age or use of inhaled corticosteroids. In conclusion, these data support a potential role for HMGB1 as a biomarker and diagnostic tool for the differential diagnosis of asthma and COPD. The importance of this observation on asthma and COPD mechanisms and outcomes should be further investigated in large prospective studies.

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are obstructive airway diseases characterized by chronic inflammation of the respiratory tract, but the type of inflammation is markedly different between them, since each has different patterns of inflammatory cells and mediators (1). Asthma is more commonly associated with Th2-mediated eosinophilic inflammation, whereas in COPD, neutrophilic inflammation is more predominant (2).

High mobility group protein B1 (HMGB1) is an abundant chromatin protein that acts as a cytokine when released in the extracellular milieu by necrotic and inflammatory cells (3). Extracellular HMGB1 can be regarded as a signal of tissue injury and a mediator of inflammation (4). High levels of HMGB1 are found in inflammatory conditions such as sepsis, cystic fibrosis and rheumatoid arthritis (5–7). Recently, Ferhani *et al.* (8) reported that levels of HMGB1 in the fluid from bronchoalveolar lavage were

also elevated in patients with COPD. Straub *et al.* (9) reported that HMGB1 inhibitors significantly diminished the ovalbumin-induced increase in response to methacholine in a mouse asthmatic model sensitized and challenged with ovalbumin. These reports suggest that HMGB1 may be an important mediator of asthma and COPD. However, the induced sputum and plasma concentrations of HMGB1 have not been systematically evaluated in patients with asthma and COPD.

We hypothesize that HMGB1 expression is increased in asthma and COPD and is related to disease severity. To test our hypothesis, we measured HMGB1 levels in induced sputum and plasma from control subjects, and patients with untreated asthma and COPD during the stable period.

MATERIALS AND METHODS

Subjects

We selected 61 asthmatic patients and 47 COPD patients from the Department of Respiratory Medicine of Southern Medical University, Nanfang Hospital (Guangzhou, China). The asthmatic patients were initially diagnosed in our hospital according to the Global Initiative for Asthma (GINA) guidelines (10). Inclusion criteria included patients or participants who had not taken corticosteroids (oral or injected), nonsteroidal antiinflammatory medications (cromolyn, ketotifen and leukotriene receptor antagonists), long-acting β2 agonists or aminophylline 3 months before this study. Exclusion criteria included (a) respiratory tract infection characterized by purulent sputum and infiltration based on x-ray or computed tomographic scans of the lungs within the previous 6 wks; (b) a history of any other lung disease except asthma; and (c) other diseases with increased levels of HMGB1 (sepsis, cystic fibrosis, rheumatoid arthritis). Patients with stable COPD were distributed between the stages of the disease according to their forced expiratory volume in the first second (FEV_1), according to the

Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (stage I, mild COPD: $FEV_1 \geq 80.0\%$ predicted; stage II, moderate COPD: $50.0\% \leq FEV_1 < 80.0\%$ predicted; stage III, severe COPD: $30.0\% \leq FEV_1 < 50.0\%$; stage IV, very severe COPD: $FEV_1 < 30.0\%$ or $FEV_1 < 50.0\%$ predicted with respiratory failure) (11). Exclusion criteria for COPD patients were similar to those in patients with asthma. A total of 34 healthy volunteers (control subjects) included patients without asthma, COPD, sepsis, cystic fibrosis or rheumatoid arthritis. The study was approved by the ethics committee of Southern Medical University, and all subjects and patients provided informed consent for participation.

Pulmonary Function Tests

Spirometry was performed using the Jaeger Masterscope® spirometry system (Jaeger, Wuerzburg, Germany) according to American Thoracic Society guidelines (12).

Asthma Patients' Symptom Score

Asthma patients were instructed to record daytime symptoms of cough, chest tightness, wheezing, sputum production and breathlessness, grading each from 0 to 3 (no symptoms = 0, mild = 1, moderate = 2, severe = 3) (13), and nocturnal symptoms for the preceding 24 h by recording how many times they woke up with asthma symptoms, grading each from 0 to 3 (none = 0, once = 1, more than once = 2, awake all night = 3) (14).

Medical Research Council (MRC) Scale

COPD patients were instructed to read the 5-point Medical Research Council (MRC) dyspnoea scale (for which higher scores represent more breathlessness) and to record the grade that most closely matched their breathlessness (15).

Blood Sample Preparation

Blood was drawn, handled and stored by the same researcher in the same department. Blood samples were collected aseptically in ethylenediamine tetraacetic

acid (EDTA)-anticoagulated tubes and stored at 4°C. Blood samples were centrifuged at 1,000*g* at 4°C for 15 min and were stored in microfuge tubes at –80°C until the measurements were taken.

Sputum Induction and Processing

For sputum induction and processing, we used the guidelines suggested by the Task Force on Induced Sputum of the European Respiratory Society (16). Subjects and patients were told to periodically spit saliva into one container and expel sputum into another. The sputum was weighed and diluted 4× in fresh 0.1% dithiothreitol (Sigma-Aldrich, St. Louis, MO, USA) in distilled water. This suspension was shaken in a vortex mixer for a few seconds and incubated in a shaking water bath at 37°C (150 cycles/min) for 15 min with aspiration every 5 min for homogenization. Samples were centrifuged at 750*g* for 10 min. The supernatant was aspirated and stored, and total cell number and cell viability were determined by the trypan blue dye exclusion method in a Neubauer chamber. Slides were prepared for differential cell counts by cytospin staining with hematoxylin-eosin.

HMGB1 Measurement

Plasma and sputum HMGB1 were quantified using a commercial HMGB1 enzyme-linked immunosorbent assay (ELISA) kit (Hyperheal, Shanghai, China). The detection limit of these kits is 0.03 ng/mL for HMGB1. Each sample was run in duplicate and compared with a standard curve. The mean concentration was determined for each sample.

Statistical Analyses

The Statistical Package for Social Sciences (SPSS), version 13.0, was used to analyze the data. Results were expressed as median (range) unless otherwise specified. Significant variation in the data within groups was investigated using the Kruskal-Wallis test. The Mann-Whitney *U* test (with Bonferroni correction) was used to compare the difference between two groups unless otherwise specified.

Table 1. Patient and subject demographics.

Characteristic	Control subjects	Asthma patients	COPD patients	*P*
n	34	61	47	
Sex (M/F)	22/12	34/27	36/11	0.199
Age (years)	44 (25–70)	39 (25–56)	60 (42–76)[a,b]	0.000
BMI	22.13 ± 4.35	22.08 ± 2.80	20.96 ± 3.44	0.184[c]
Never/current/ex-smokers	29/5/0	42/7/12	18/11/18	
Smoking history (pack-years)	20 (12.5–35)	15 (2.5–50)	45 (20–100)[a,b]	0.012
Family asthma history (Y/N)	4/30	16/45[a]		0.000
Allergic rhinitis (Y/N)	3/31	19/42[a]		0.000
Inhaled corticosteroid (Y/N)	0/34	0/61	26/21	
Short-acting β agonist (Y/N)	0/34	0/61	22/25	
Aminophylline (Y/N)	0/34	0/61	15/32	
Long-acting β2 agonist (Y/N)	0/34	0/61	20/27	
Sputum cell counts				
TCC (10^6/mL)	3.55 (1.62–6.60)	4.20 (1.74–11.36)	7.50 (3.06–13.20)[a,b]	0.000
Macrophages (%)	52.5 (31.0–80.0)	41 (8.0–90.50)	30 (2.0–56.0)[a,b]	0.000
Neutrophils (%)	43.75 (20.0–65.0)	37.5 (1.5–70.0)	67.5 (43.0–88.5)[a,b]	0.000
Lymphocytes (%)	2.0 (0.0–12.0)	3.0 (0.0–24.5)	2.0 (0.0–14.0)	0.080
Eosinophils (%)	1.0 (0.0–8.0)	9.0 (0.0–83.0)[a]	1.0 (0.0–2.5)[b]	0.000
FVC (L)	3.51 (1.72–5.82)	2.84 (1.36–4.57)[a]	1.92 (1.18–5.01)[a,b]	0.000
PEF (L/s)	7.97 (4.72–12.10)	5.45 (1.42–10.75)[a]	1.92 (2.5–50)[a,b]	0.000
FEV_1 (L)	3.07 (2.00–4.14)	2.20 (0.57–4.09)[a]	0.78 (0.47–1.91)[a,b]	0.000
FEV_1% predicted	102.70 (88.00–121.90)	75.10 (24.40–116.30)[a]	34.00 (15.60–86.00)[a,b]	0.000
Daily score		6 (1–12)		
Nighttime score		1 (0–3)		
MRC dyspnoea scale			3 (1–5)	
Peripheral blood counts				
TCC (10^9/mL)	7.31 (5.58–8.21)	6.74 (4.41–16.38)	6.76 (2.93–17.09)	0.801
Lymphocytes (%)	35.60 (28.20–42.80)	27.40 (6.80–53.20)	17.20 (3.20–44.40)[a,b]	0.000
Neutrophils (%)	56.30 (48.20–60.90)	60.40 (35.10–85.40)	73.50 (48.30–84.20)[a,b]	0.000
Macrophages (%)	6.95 (4.70–7.20)	5.90 (1.25–17.20)	7.60 (0.40–17.70)	0.054
Eosinophils (%)	2.10 (1.40–2.90)	5.10 (0.00–9.40)[a]	1.69 (0.00–10.10)[b]	0.000

Data are medians (range), except for BMI, which are means ± SEM. TCC, total cell count; FVC, forced vital capacity; PEF, peak expiratory flow.
[a]*P* < 0.05 versus control group; [b]*P* < 0.05 versus asthma group.
[c]One-way ANOVA and independent sample *t* test were performed.

Frequency data (for example, sex) were analyzed by the χ^2 test. For the comparison of HMGB1 among all groups of subjects, a univariate general linear model (analysis of covariance [ANCOVA]) adjusting for confounding factors (sex, age, smoking habit, inhaled corticosteroids [ICS] use and pulmonary function index) was used (17). Correlations were assessed by Spearman rank correlation coefficients. Multiple regressions analysis was performed for the evaluation of HMGB1 predictors, using HMGB1 in plasma or induced sputum as dependent variables; sex, age, smoking pack-years, the use of long-acting β2 agonists, ICS,

aminophylline, short-acting β agonists, and asthma/COPD and FEV_1% predicted were used as independent variables. Stepwise multiple regression analysis was also used to develop a prediction equation for HMGB1 level using significant parameters after the initial multiple regression analysis. *P* values <0.05 were considered statistically significant.

RESULTS

Demographic characteristics of the patients with COPD and asthma and control subjects are shown in Table 1. There were no significant differences between

patients and subjects in sex and body mass index (BMI), whereas median age, the number of smokers and smoking pack-years were significantly greater in COPD patients compared with asthma patients and control subjects (*P* < 0.001). In the asthma patient group, the number of patients with a family history of asthma (16/45 patients) and allergic rhinitis (19 of 42 patients) was significantly higher compared with healthy controls (4/30 patients and 3/31 patients, respectively). The total number of white blood cells in induced sputum not in the peripheral blood of COPD patients was significantly higher than that in

A

B
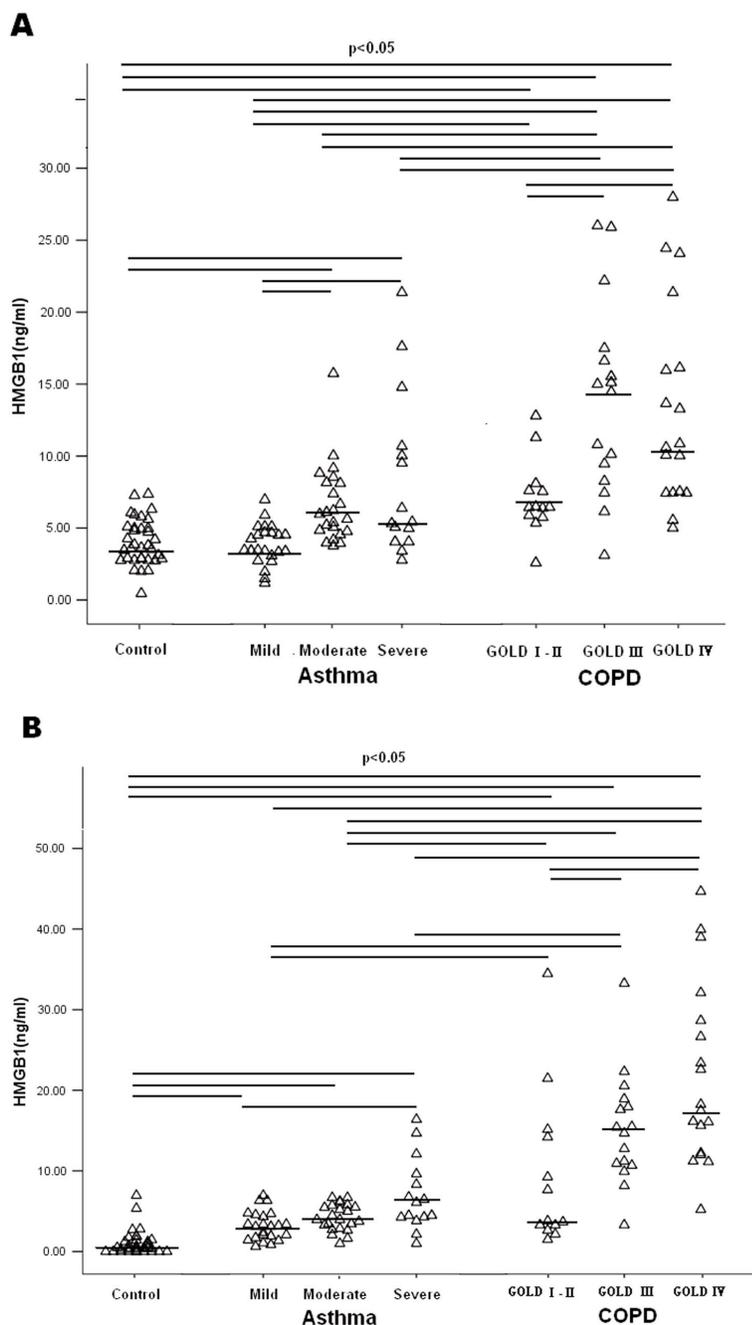
Figure 1. (A) HMGB1 concentration in plasma from asthma patients and COPD patients of different severity. (B) HMGB1 concentration in induced sputum from healthy controls, asthma patients and COPD patients of different severity. Kruskal-Wallis test (within groups) and Mann-Whitney *U* test (between groups; with Bonferroni correction) were used. The line represents the median.

asthma patients and healthy controls (*P* < 0.001). COPD patients had a higher percentage of sputum and blood neutrophils and lower percentage of blood

lymphocytes than asthma patients (*P* < 0.05) and healthy controls (*P* < 0.05). In contrast, compared with COPD patients or with healthy controls, patients with

asthma had a significantly (*P* < 0.05) greater percentage of blood eosinophils. The disease severity of COPD patients we recruited was greater than the disease severity of patients with asthma (*P* < 0.001).

Differences in HMGB1 between all subgroups of controls, COPD patients and asthma patients are presented in Figure 1. Compared with controls (0.41 [0-6.95]), HMGB1 levels in induced sputum were significantly higher (*P* < 0.001) in patients with all severities of asthma (4.47 [0.98-16.39]) and those with COPD (15.15 [1.49-44.68]). Similarly, compared with controls, plasma HMGB1 levels were significantly higher in patients with moderate (n = 23, *P* < 0.001) and severe (n = 15, *P* = 0.012) asthma and in those with COPD GOLD stage I and II combined (n = 13, *P* < 0.001), stage III (n = 16, *P* < 0.001) and stage IV (n = 18, *P* < 0.001). The plasma and sputum HMGB1 levels were significantly higher (*P* = 0.006 and *P* = 0.012, respectively) in patients with severe asthma than in patients with mild asthma. Plasma HMGB1 levels were significantly higher in patients with moderate asthma than in patients with mild asthma (*P* < 0.001). Similarly, plasma HMGB1 levels were higher in patients with COPD GOLD stage III (*P* = 0.006) and stage IV (*P* = 0.0024) compared with patients with COPD GOLD stage I and II combined (Figure 1A); sputum HMGB1 levels were higher in patients with COPD GOLD stage III (*P* = 0.017) and stage IV (*P* = 0.006) than in patients with COPD GOLD stage I and II combined (Figure 1B). After adjusting for sex, age, smoking, the use of long-acting β2 agonists, ICS, aminophylline and short-acting β agonists, all the differences presented in Figure 1 were statistically significant. Again, there was no significant difference in sputum HMGB1 levels between patients with mild asthma and health controls (*P* = 0.170) and between mild asthma and moderate asthma (*P* = 0.412).

HMGB1 levels in plasma and induced sputum of COPD patients were signifi-

Table 2. HMGB1 levels in plasma and induced sputum of healthy subjects, noneosinophilic asthma patients and eosinophilic asthma patients.

Group	n	Plasma (ng/mL)	Induced sputum (ng/mL)
Control	34	3.71 (0.45–7.35)	0.41 (0–6.95)
Noneosinophilic asthma	20	6.30 (1.96–21.36)[a]	4.57 (1.05–14.68)[b]
Eosinophilic asthma	41	4.73 (1.18–15.74)[a]	3.80 (1.02–16.39)[b]
P		0.002	<0.001

Data are medians (range).
[a]$P < 0.05$ versus control group.
[b]$P < 0.001$ versus control group.

cantly higher than levels in asthma patients ($P < 0.001$). Also sputum and plasma HMGB1 concentrations were greater in the subjects with COPD GOLD stage II–IV combined compared with subjects with moderate-severe asthma combined ($P < 0.001$). Moreover, after adjusting for previously mentioned variables and pulmonary function, patients with COPD still showed significantly greater concentrations of sputum HMGB1 than HMGB1 concentrations in patients with all severities of asthma ($P < 0.05$); however, there were no statistically significant differences in plasma concentration of HMGB1 between these patients ($P = 0.561$).

Although patients with noneosinophilic asthma had higher plasma and sputum HMGB1 levels compared with eosinophilic asthma patients, the differences were not significant ($P = 0.132$ and $P = $

0.990, respectively; Table 2). A cutoff point of 3% eosinophils in induced sputum was applied to distinguish eosinophilic from noneosinophilic asthma patients (18).

HMGB1 levels in plasma and induced sputum in all subjects and patients showed a significant negative correlation with lung function parameters such as FEV_1, FEV_1 (% predicted) and FEV_1/FVC (forced vital capacity) ratio. In all participants, compared with plasma HMGB1 levels, sputum HMGB1 levels showed stronger negative correlation with pulmonary function index (Tables 3 and 4).

Predictors of HMGB1 in plasma were provided by multiple regression analysis.

The results showed that disease severity, but not sex, age, smoking habit, the use of long-acting β2 agonists, ICS, aminophylline, short-acting β agonist, and diseases groups, was the indepen-

dent predictor of HMGB1 (R^2 0.402, adjusted R^2 0.361). Stepwise multiple regression analysis using FEV_1% predicted as independent variables showed that HMGB1 values in plasma could be predicted on the basis of FEV_1% predicted (unadjusted R^2 0.300, adjusted R^2 0.295; Table 5). Also, disease severity and disease groups were the predictors of HMGB1 levels in sputum (R^2 0.576, adjusted R^2 0.564). Stepwise multiple regression analysis using FEV_1% predicted and disease groups as independent variables showed that HMGB1 in induced sputum could be predicted on the basis of FEV_1% predicted and disease groups (unadjusted R^2 0.494, adjusted R^2 0.487; Table 6).

Plasma and induced sputum HMGB1 levels in all 142 subjects, sputum HMGB1 levels from the COPD group and plasma from the asthma group were all positively correlated with neutrophil counts and percentage of neutrophils. Sputum HMGB1 levels from COPD patients were positively correlated with macrophage count. In COPD patients but not in asthma patients, sputum HMGB1 levels were negatively correlated with eosinophil counts and percentage of eosinophils. Sputum HMGB1 levels were positively correlated with plasma levels in control subjects as well as patients in the asthma group, but not patients in the COPD group. In addition, HMGB1 levels

Table 3. Correlations between HMGB1 concentration and lung function and cells in induced sputum and peripheral blood in all study subjects.

	Index	r_s	P		Index	r_s	P
P-HMGB1	FVC	–0.617	0.000	S-HMGB1	FVC	–0.697	0.000
P-HMGB1	PEF	–0.656	0.000	S-HMGB1	PEF	–0.789	0.000
P-HMGB1	FEV_1	–0.637	0.000	S-HMGB1	FEV_1	–0.791	0.000
P-HMGB1	FEV_1% predicted	–0.602	0.000	S-HMGB1	FEV_1% predicted	–0.755	0.000
P-HMGB1	B-neu%	0.466	0.000	S-HMGB1	S-neu%	0.547	0.000
P-HMGB1	B-neu	0.409	0.001	S-HMGB1	S-neu	0.547	0.000
P-HMGB1	B-mon	0.185	0.051	S-HMGB1	S-mon	0.179	0.033
P-HMGB1	B-eos%	–0.416	0.000	S-HMGB1	S-eos%	–0.095	0.260
P-HMGB1	B-eos	–0.411	0.000	S-HMGB1	S-eos	0.056	0.508
P-HMGB1	S-HMGB1	0.613	0.000				

Study subjects (N = 142). Data are Spearman rank correlation coefficients. P-HMGB1, HMGB1 level in plasma; S-HMGB1, HMGB1 level in sputum; FVC, forced vital capacity; PEF, peak expiratory flow; B-neu, neutrophils in peripheral blood; B-mon, macrophages in peripheral blood; B-eos, eosinophils in peripheral blood; S-neu, neutrophils in induced sputum; S-mon, macrophages in induced sputum; S-eos, eosinophils in induced sputum.

Table 4. Correlations between HMGB1 concentration and lung function, cells in induced sputum and peripheral blood and symptom score in asthma and COPD groups.

Asthma (n = 61)				COPD (n = 47)			
	Index	r_s	P		Index	r_s	P
P-HMGB1	FVC	–0.299	0.019	P-HMGB1	FVC	–0.468	0.001
P-HMGB1	PEF	–0.432	0.001	P-HMGB1	PEF	–0.360	0.013
P-HMGB1	FEV_1	–0.371	0.003	P-HMGB1	FEV_1	–0.337	0.021
P-HMGB1	FEV_1% predicted	–0.289	0.024	P-HMGB1	FEV_1% predicted	–0.222	0.133
P-HMGB1	B-neu%	0.246	0.056	P-HMGB1	B-neu%	0.323	0.027
P-HMGB1	B-neu	0.418	0.001	P-HMGB1	B-neu	0.232	0.117
P-HMGB1	B-mon	0.188	0.146	P-HMGB1	B-mon	0.022	0.881
P-HMGB1	B-eos%	–0.076	0.562	P-HMGB1	B-eos%	–0.460	0.001
P-HMGB1	B-eos	–0.062	0.635	P-HMGB1	B-eos	–0.428	0.003
P-HMGB1	Nighttimescore	0.300	0.019	P-HMGB1	MRC dyspnoea scale	0.021	0.887
P-HMGB1	S-HMGB1	0.341	0.007	P-HMGB1	S-HMGB1	0.172	0.248
S-HMGB1	FVC	–0.466	0.000	S-HMGB1	FVC	–0.355	0.014
S-HMGB1	PEF	–0.468	0.000	S-HMGB1	PEF	–0.613	0.000
S-HMGB1	FEV_1	–0.442	0.000	S-HMGB1	FEV_1	–0.590	0.000
S-HMGB1	FEV_1% predicted	–0.369	0.003	S-HMGB1	FEV_1% predicted	–0.506	0.000
S-HMGB1	S-neu%	–0.012	0.925	S-HMGB1	S-neu%	0.321	0.028
S-HMGB1	S-neu	0.044	0.631	S-HMGB1	S-neu	0.492	0.000
S-HMGB1	S-mon	0.134	0.736	S-HMGB1	S-mon	0.352	0.015
S-HMGB1	S-eos%	–0.023	0.861	S-HMGB1	S-eos%	–0.204	0.169
S-HMGB1	S-eos	0.004	0.978	S-HMGB1	S-eos	–0.057	0.702
S-HMGB1	Nighttime score	0.231	0.073	S-HMGB1	MRC dyspnoea scale	–0.564	0.000

Data are Spearman rank correlation coefficients. P-HMGB1, HMGB1 level in plasma; S-HMGB1, HMGB1 level in sputum; FVC, forced vital capacity; PEF, peak expiratory flow; B-neu, neutrophils in peripheral blood; B-mon, macrophages in peripheral blood; B-eos, eosinophils in peripheral blood; S-neu, neutrophils in induced sputum; S-mon, macrophages in induced sputum; S-eos, eosinophils in induced sputum.

in sputum, but not in plasma, correlated positively with the MRC dyspnoea scale in the COPD group. In the asthma group patients, plasma HMGB1 levels correlated positively with the nighttime score but not with the daily score (see Tables 3 and 4).

DISCUSSION

This is the first report showing that sputum HMGB1 concentrations in patients with COPD and plasma HMGB1 concentrations in patients with asthma were greater than concentrations in control subjects and correlated with patients' disease severity; we also show that the concentrations of HMGB1 positively and significantly correlated with neutrophil counts and percentage of neutrophils; finally, consistent with recent findings by Watanabe *et al.* (19), our report confirmed that HMGB1 concentrations in induced sputum were elevated in asthma patients and negatively correlated with lung func-

tion parameters. Also, consistent with the findings by Shang *et al.* (20), we showed that plasma HMGB1 levels in COPD patients were significantly greater than plasma HMGB1 levels in healthy controls.

To the best of our knowledge, this is the first report comparing HMGB1 concentrations in patients with asthma and patients with COPD. The distinct cellular and molecular expression features of airways inflammation in COPD and asthma patients offers opportunities to distinguish between these two diseases (21). Compared with asthma patients, patients with COPD showed significantly increased induced sputum HMGB1 levels, even after adjustments for age, smoking habit, the use of ICS and disease severity. The fact that disease severity was an independent predictor of plasma and sputum HMGB1 further supports a potential role for HMGB1 as a new biomarker for asthma and COPD. Further, because the diseases (asthma and COPD) were inde-

pendent predictors of HMGB1 in sputum supports the hypothesis that HMGB1 could be an important biomarker in diagnosing asthma and COPD.

In a previous study (22), we showed that compared with control mice, mice with respiratory syncytial virus (RSV) showed increased expression and release of HMGB1 in lung bronchoalveolar lavage fluid (BALF), suggesting that HMGB1 is involved in the development of RSV infection–related lung diseases such as acute asthma. We also showed that the expression of HMGB1 is increased in the lung and BALF of asthmatic mice (23), and dexamethasone does not significantly alter HMGB1 expression. This study further demonstrated that HMGB1 levels in plasma and sputum were elevated in asthmatic patients. In addition, HMGB1 enhances the survival and chemotaxis of eosinophils (24). Several studies (9,19,22,23) support an important role of HMGB1 in the de-

Table 5. Prediction equations for HMGB1 level in plasma.

	B[a]	SE	β[b]	95% CI for B	P
Constant	14.135	0.947		12.262 to 16.008	0.000
FEV$_1$% predicted	−0.101	0.013	−0.548	−0.126 to −0.075	0.000

[a]Unstandardized coefficients.
[b]Standardized coefficients.
Dependent variable: HMGB1 level in plasma.

Table 6. Prediction equations for HMGB1 level in induced sputum.

	B[a]	SE	β[b]	95% CI for B	P
Constant	6.525	4.133		−1.647 to 14.697	0.117
FEV$_1$% predicted	−0.116	0.029	−0.393	−0.172 to −0.059	0.000
Asthma/COPD	4.169	1.157	0.351	1.881 to 6.457	0.000

[a]Unstandardized coefficients.
[b]Standardized coefficients.
Dependent variable: HMGB1 level in induced sputum.

velopment of asthma. Noneosinophilic and eosinophilic asthma are likely produced by different immunological mechanisms, including a difference in cytokine production (25). Accordingly, we compared the concentration of HMGB1 in these two phenotypes. However, in this study, we found no significant differences in HMGB1 levels between noneosinophilic asthma and eosinophilic asthma patients, a finding that needs further investigation. Our finding could be partly explained by the fact that there were different sources of HMGB1 such as from eosinophils, macrophages and neutrophils (1,8,26).

To explore the role of HMGB1 in non–small-cell lung cancer (NSCLC), plasma HMGB1 levels were analyzed by Western blot analysis in patients with NSCLC, in patients with COPD (excluding lung cancer), and in healthy volunteers (20). The results showed a significant increase in the HMGB1 concentrations from COPD patients in the stable period compared with healthy controls (21). In another study by Ferhani *et al.* (8), HMGB1 assessed in BALF of 20 nonsmokers, 20 smokers and 30 smokers with COPD showed that BALF levels of HMGB1 were elevated and correlated positively with interleukin (IL)-1β and negatively with FEV$_1$. These two observations also suggest that HMGB1 participates in the pathogenesis of COPD.

It has been demonstrated that induced sputum is more concentrated and richer in airway secretions than BALF (27). Samples from induced sputum are valid in assessing diseases involving airway inflammation, and sputum testing is safe and noninvasive. Although our results need further clarification, they indicate that HMGB1 in induced sputum may be a novel and useful biomarker for reflecting disease severity of asthma and COPD.

Our finding that HMGB1 in induced sputum correlated positively with neutrophil count and percentage of neutrophils in all participants is also supported by the finding that neutrophils contribute to increased levels of HMGB1 (28) and a study showing that purified HMGB1 can stimulate dose-dependent chemotaxis of neutrophils (6). These findings suggest that neutrophils may be an important source of HMGB1, and a positive feedback loop may exist between HMGB1 and neutrophils, an area we intend to explore in future studies. It was also recently reported (8) that elevated numbers of alveolar macrophages may increase HMGB1 levels in BALF from smokers with COPD, which is consistent with our finding that the number

of macrophages correlated positively with HMGB1 levels in the sputum from COPD patients. Although Straub found that eosinophils could express and actively release HMGB1 upon activation with granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α or interferon (IFN)-γ (26), we found no significant correlation between HMGB1 and eosinophils in the asthma group. In our study, HMGB1 levels in COPD patients showed a negative correlation with eosinophil counts and percentages of eosinophils, and it remains to be established whether eosinophils can release HMGB1 in asthma and COPD patients. Ferhani *et al.* (8) also reported that bronchial epithelial cells are a potential source of HMGB1 in the airways of COPD patients, which is consistent with one previous study (29) and another (unpublished data) showing that hydrogen peroxide, but not lipopolysaccharide, can induce the expression and release of HMGB1 from bronchial epithelial cells *in vitro*.

In our study, we could not exclude the effects of smoking, age and different medications used by the patients with COPD and asthma. In healthy subjects, HMGB1 levels do not appear to be associated with age (31), and our results showed that there were no significant differences in levels of plasma and induced sputum HMGB1 between nonsmoking and smoking control subjects as well as in asthma and COPD patients, a finding that is consistent with results reported by Fukami *et al.* (30) and Ferhani *et al.* (8); also, the results of multiple regression analysis showed that sex, age, smoking habit and medicine usage were not the independent predictors of HMGB1 levels. Nevertheless, rats exposed to cigarette smoke show increases in serum HMGB1 levels compared with rats not exposed to cigarette smoke (31). Thus, further studies will be needed to clarify whether cigarette smoke affects the expression of HMGB1 in patients with asthma and COPD.

Understanding the role of HMGB1 in asthma and COPD is complex, and much

remains to be clarified. HMGB1 displays a wide range of immunological effects in addition to its cytokine effects, and it can be considered an alarm that alerts our defense system of an impending danger (32–34). Therefore, we speculate that HMGB1 may be an important mediator for asthma and COPD. However, we did not study whether HMGB1 could predict disease activity or how it might participate in disease pathogenesis, which underscores the need to further evaluate and define the precise role HMGB1 plays in the pathogenesis of asthma and COPD.

However, although the strength of the study is the careful selection of patients without comorbidities, this study has limitations. One significant limitation is that there were significant demographic differences between the three groups in age, smoking status and disease severity. COPD patients were older than other patients and controls. Also, compared with asthma patients and healthy controls, patients with COPD had a significantly greater number of smoking pack-years and, compared with asthma patients, had greater disease severity. However, these differences may be in part attributed to the prevalence of the two diseases (35,36). It is known that COPD is more prevalent in elderly patients, and 61.4% of the patients with COPD were smokers in China (35); however, the average age of asthma patients in our previous study including 120 patients was about 40 years, and about 25.0% of them were smokers (36). Also, we found that compared with COPD patients or with healthy controls, patients with asthma had a significantly ($P < 0.05$) greater percentage of blood eosinophils and that the disease severity of COPD patients we recruited was greater than the disease severity of patients with asthma ($P < 0.001$). These two findings are consistent with a UK study (37). Moreover, the fact that most of the significant differences in HMGB1 between our study groups remained after our additional analysis in which we adjusted for the study confounders further supports the need for

evaluation of HMGB1 in patients with asthma and COPD.

In conclusion, our finding that increased induced sputum and circulating HMGB1 levels in patients with asthma and COPD is associated with disease severity supports the hypothesis that HMGB1 may play an important role in the development of asthma and COPD and suggests that HMGB1 antagonists may be an attractive alternative therapy for patients with asthma and COPD. Further investigation will be necessary to determine the sites, mechanisms and consequences of HMGB1 in these inflammatory disorders.

ACKNOWLEDGMENTS

DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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