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ORIGINAL ARTICLE

Differences in IL-8 in serum and exhaled breath condensate from patients with exacerbated COPD or asthma attacks

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Background/Purpose: The collection of exhaled breath condensate (EBC) is a noninvasive method that can be used to monitor the inflammatory status of patients with chronic airway diseases. We aimed to study differences in cytokine expression between patients with exacerbations of chronic obstructive pulmonary disease (COPD) and patients with asthma attacks.
Methods: Using a custom-made device and methods based on American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations, EBC samples were collected from nine COPD patients, 12 asthma patients and 10 healthy individuals. Cytokine concentrations in serum and EBC were measured via commercial ELISA kits.
Results: Of four cytokines measured in EBC [interleukin-8 (IL-8), IL-17, IL-4 and tumor necrosis factor-α (TNF-α)], only IL-8 was significantly higher in COPD than in asthma patients (5.27 ± 0.18 vs. 4.36 ± 0.34 pg/mL, $p = 0.001$). Moreover, COPD patients had higher serum IL-8 than asthma patients (10.57 ± 0.55 vs. 5.15 ± 0.24 pg/mL, $p < 0.001$). No significant correlation between serum and EBC cytokine concentrations was observed in each subgroup of patients.

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Conclusion: Compared with patients with asthma attacks, patients with exacerbated COPD had increased IL-8 expression in both serum and EBC. These results suggest that IL-8 may be more important in airway and systemic inflammation in COPD exacerbations than in asthma attacks.

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic airway inflammatory disease, characterized by irreversible airflow limitation that increases progressively during the natural course of the disease. Patients with COPD may develop periods of exacerbated symptoms during their life, and these symptoms are thought to be related to acute infection.[1] Recent studies indicated that recurrent exacerbation of COPD contributes to disease progression and a poor prognosis.[2,3] There is a lack of information about the inflammatory mechanisms involved in exacerbations of COPD. It is therefore important to investigate inflammatory changes in patients with exacerbated COPD.

Exhaled breath condensate (EBC), formed through a condensing device, is a noninvasive method to analyze inflammatory biomarkers in patients with chronic pulmonary disease. Increased leukotriene B4 and 8-isoprostane in EBC of patients with exacerbated COPD has been reported in a previous study.[4] Increased interleukin-4 (IL-4) was found in EBC from children with asthma, and this correlated with the pathophysiologic characteristics of asthma.[5] Although the methods used for EBC collection and analysis vary widely, there is considerable evidence to support the use of EBC biomarkers for monitoring disease activity and the response to pharmacological intervention.[6,7]

The involving immunological mechanism and associated inflammatory mediators between asthma and COPD are different. By secreting IL-4 and other cytokines, Th2 cells play an important role in allergic airway inflammation in asthma. IL-8, a chemokine of neutrophils, is correlated with the extent of neutrophilic inflammation and disease severity in COPD.[8,9] Through analysis of induced sputum, TNF-α is also considered to be important in inflammation of COPD.[8] IL-17, a proinflammatory cytokine mainly produced by Th17 cells, is thought to induce and sustain the neutrophil-mediated inflammatory response.[10] Although increased IL-17 concentrations have been reported in the sputum of asthmatic patients,[11] little is known about the role of IL-17 in asthma or COPD.

Through measuring these cytokines in serum and EBC in patients with exacerbated COPD and in patients with asthma attacks, the goal of the present study is to characterize the difference of the inflammatory cytokine pattern between COPD exacerbations and asthma attacks.

Methods

Participants and protocol

This study enrolled nine patients with acute exacerbations of COPD and 12 patients with acute asthma attacks from Chung-Shan Medical University Hospital. Another 10 healthy volunteers were recruited from the medical staff and served as a control group. The patients with exacerbated COPD were required to have symptoms that fulfilled the presentation defined by the guidelines from the Global Initiative for Chronic Obstructive Lung Disease.[1] The definition of acute asthma attack was according to the guidelines from the Global Initiative for Asthma.[12] The healthy participants had no history of smoking, no chronic underlying diseases, no recent upper airway infection and no chronic respiratory symptoms (e.g., chronic cough, productive cough, wheezing, etc.).

Patients with exacerbated COPD received both antibiotic treatment (oral or intravenous amoxicillin/clavulanic acid for 3—5 days) and systemic steroids during admission, but patients with asthma attacks received systemic steroids only. All patients and healthy volunteers completed a detailed questionnaire on their personal medical history. We excluded patients with concomitant pneumonia, patients with severe respiratory distress, patients with severe hypoxemia and patients with an unstable hemodynamic status. The protocol was approved by the Institutional Review Board of the hospital, and written informed consent was obtained from all participants.

Collection of exhaled breath condensate

EBC was collected with a custom-made condensing device, and the collection time was 10 minutes when participants were breathing at their tidal volume. The design of the condensing device and collection methods were based on recommendations by the American Thoracic Society/European Respiratory Society task force on EBC.[13] The details of the custom-made EBC collecting device are illustrated in Fig. 1. It consisted of a Liebig condenser, a pump that recycled cold water, a glass tube connector, a saliva trap and two one-way valves. The condenser and its inner surface were made of glass. Ice cold water (4°C) was used for cooling the condenser. The two one-way vales prevented the patient from inhaling cold air that had passed through the condenser during inspiration. The saliva trap was designed to avoid saliva contamination during EBC collection. There was no resistor or filter between the participants and the condenser. Nose clips were not used during EBC sampling, due to possible discomfort in patients with obstructive airway diseases. The EBC collections were done on Day 1 or Day 2 after hospitalization for patients with exacerbated COPD or asthma attacks. To prevent the influence of smoking on cytokine levels, patients who smoked were advised to refrain from smoking for at least 12 hours before EBC collection. The EBC samples were stored in 1.5 mL microcentrifuge tubes and frozen at −70°C immediately until further assay.

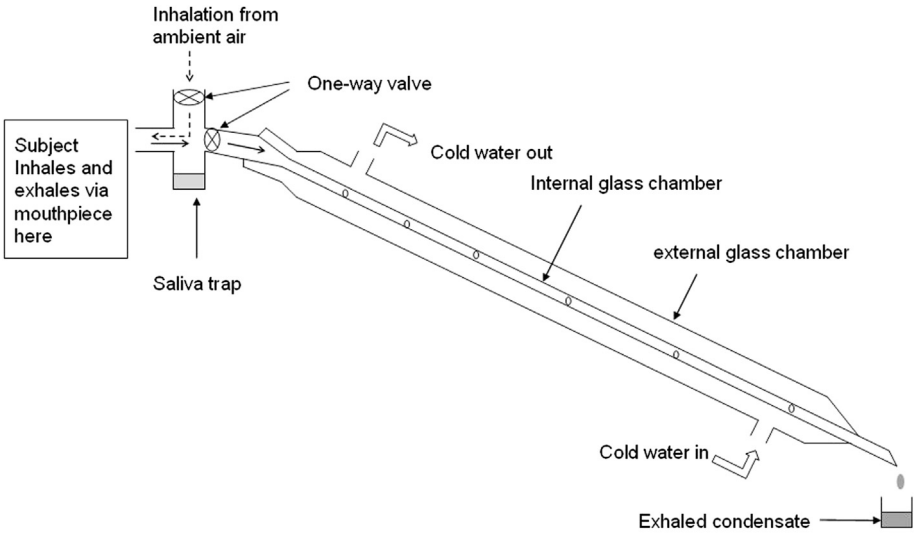

Figure 1 Schematic diagram of exhaled breath condensate (EBC) collecting device.

Serum samples

Blood samples from patients with exacerbated COPD and asthma attacks were drawn at the time when patients completed EBC collections, clotted at 4°C for 60 minutes and then centrifuged for 30 minutes at 3000 rpm. Serum samples were frozen at −70°C immediately until further assay.

Measurement of cytokine levels

The serum and EBC concentrations of IL-8, IL-17, IL-4, and TNF-α were determined using commercially quantitative enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, San Diego, CA) according to the manufacturer's instructions. However, there were a few deviations from the manufacturer's instructions in order to increase the sensitivity of the assays. Processes including coating with capture antibodies, blocking, incubation of samples, and reaction with detecting antibodies, were performed at 4°C, and the reaction time was prolonged to 8 hours for enhancing the assay sensitivity.

Intrasubject reproducibility of measurement was assessed by collection of EBC in three healthy participants and comparison of EBC cytokine concentrations within the same individual. EBC samples were collected consecutively three times in each healthy participant on the same day. The cytokine concentrations in these samples were determined via the same ELISA plates. The average intrasubject coefficients of variation were 6.6% and 7.5% for IL-8 and TNF-α measurement, respectively.

Statistical analysis

Demographic and clinical data are expressed as the mean ± SD, and cytokine data as the mean ± SEM. A Mann-Whitney U-test and a Kruskal-Wallis test were used to compare values among different groups. Correlations between serum and EBC cytokine concentrations were determined using Spearman's rank correlation test. A p value <0.05 was considered significant. All analyses were performed using the SPSS Rel. 12.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

The demographic data, lung function and volume of EBC collected from all participants are shown in Table 1. Compared with the exacerbated COPD patients, patients

Table 1 Demographic data of all participants.

	COPD (n = 9)	Asthma (n = 12)	Control (n = 10)	p
Age (y)	72 ± 4.7	48 ± 8.5	44 ± 8.5	0.002
Females/ males	0/9	7/5	5/5	0.019
Weight (kg)	60 ± 7	63 ± 7	66 ± 4	0.422
Height (cm)	164 ± 3.9	162 ± 5.5	165 ± 4.7	0.931
FEV1/FVC ratio	0.62 ± 0.10	0.64 ± 0.07	—	0.651
FEV1 (L)	1.29 ± 0.52	1.58 ± 0.55	—	0.356
FVC (L)	2.16 ± 0.63	2.53 ± 0.82	—	0.337
EBC volume (μL)	703 ± 213	742 ± 230	1343 ± 442	0.145
Smoking history				0.001
Non-smokers	0	7	10	
Smokers	9	5	0	

COPD: patients with exacerbated COPD.
Asthma: patients with asthma attacks.
Control: healthy individuals.
COPD = chronic obstructive pulmonary disease; EBC = exhaled breath condensate; FEV1 = forced expiratory volume in 1 second; FVC = forced vital capacity.

with asthma attacks and healthy volunteers were younger ($p = 0.002$) and had a higher ratio of females to males ($p = 0.019$). The smoking status between these three groups was significantly different. No significant differences were found between the COPD and asthma patients in forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) ratio, FEV1, FVC, collected EBC volume (703 ± 213 vs. 742 ± 230 μL), body weight and height.

Cytokine concentrations in EBC

EBC measurements of IL-8 and IL-17 were performed in patients with exacerbated COPD, patients with asthma attacks and healthy participants. Because the EBC volume was not sufficient in several patients' samples, IL-4 was measured only in patients with asthma attacks and healthy participants, and TNF-α was measured only in patients with exacerbated COPD and healthy participants.

The EBC IL-8 concentrations were significantly higher in patients with exacerbated COPD than in patients with asthma attacks (5.27 ± 0.18 vs. 4.36 ± 0.34 pg/mL, $p = 0.001$) (Fig. 2A). Patients with asthma attacks also had lower EBC IL-8 concentrations than healthy individuals (4.36 ± 0.34 vs. 4.90 ± 0.05 pg/mL, $p = 0.001$). There was no significant difference in EBC IL-8 expression between patients with exacerbated COPD and healthy individuals ($p = 0.061$). The EBC IL-17 concentrations were not significantly different among patients with exacerbated COPD, patients with asthma attacks and healthy individuals (0.90 ± 0.27 vs. 0.50 ± 0.12 vs. 0.54 ± 0.15 pg/mL, respectively) (Fig. 2B). The EBC IL-4 concentrations were not significantly different between patients with asthma attacks and healthy individuals (0.33 ± 0.03 vs. 0.02 ± 0.02 pg/mL) (Fig. 2C). The EBC TNF-α concentrations were not significantly different between patients with exacerbated COPD and healthy individuals (0.35 ± 0.05 vs. 0.21 ± 0.04 pg/mL) (Fig. 2D).

Serum cytokine concentrations

Because serum sampling was not done in healthy participants, serum measurements of IL-8 and IL-17 were performed in patients with exacerbated COPD and patients with asthma attacks. For matching serum data with EBC data and economical concern, serum IL-4 was measured only in patients with asthma attacks. The serum TNF-α was measured only in patients with exacerbated COPD.

The serum IL-8 concentrations were also significantly higher in patients with exacerbated COPD than in patients with asthma attacks (10.57 ± 0.55 vs. 5.15 ± 0.24 pg/mL,

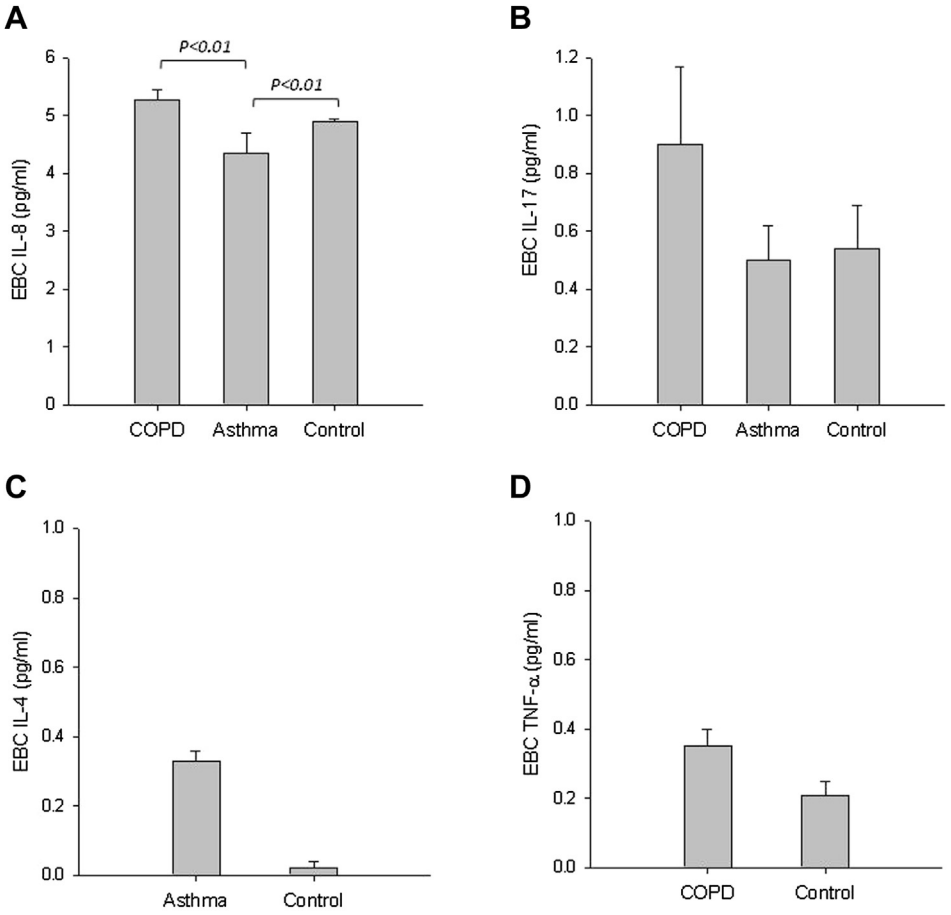

Figure 2 (A) Interleukin (IL)-8; (B) IL-17; (C) IL-4; and (D) tumor necrosis factor (TNF)-α concentrations in exhaled breath condensate (EBC) from patients with exacerbated chronic obstructive pulmonary disease (COPD) (COPD: $n = 9$), patients with asthma attack (Asthma: $n = 10–12$), and healthy individuals (Control: $n = 10$). Values are presented as mean ± SEM.

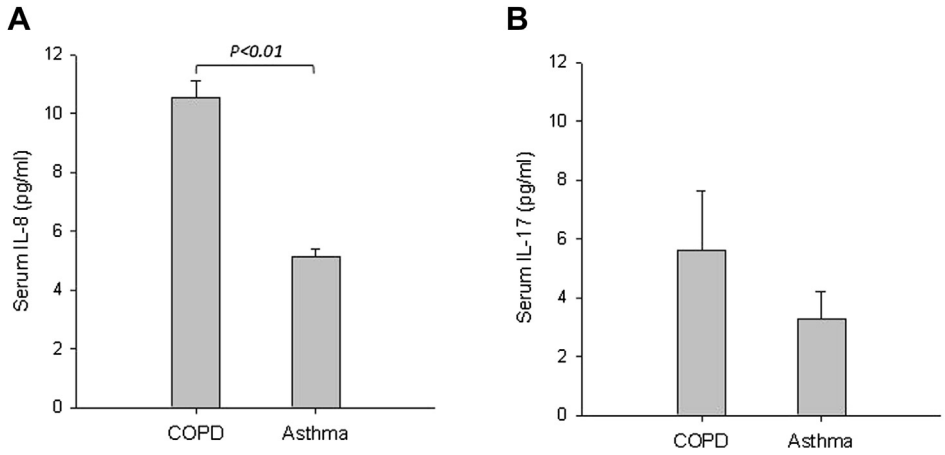

Figure 3 Serum concentrations of (A) interleukin (IL)-8; and (B) IL-17 from patients with exacerbated chronic obstructive pulmonary disease (COPD) (COPD: $n = 9$) and from patients with asthma attacks (Asthma: $n = 11-12$). Values are presented as mean ± SEM.

$p < 0.001$) (Fig. 3A). The serum IL-17 concentrations were not significantly different between patients with exacerbated COPD and patients with asthma attacks (5.61 ± 2.02 vs. 3.31 ± 0.90 pg/mL) (Fig. 3B). The values of serum IL-4 concentrations were 0.81 ± 0.17 pg/mL in patients with asthma attacks, and the values of serum TNF-α concentrations were 3.16 ± 0.77 pg/mL in patients with exacerbated COPD.

Relationship between serum and EBC cytokine levels

A correlation analysis was performed to determine the relationship between serum and EBC cytokine levels in each subgroup of patients (Table 2). There was no significant correlation between serum and EBC levels of IL-8, IL-17, IL-4 or TNF-α.

Discussion

We have shown that IL-8 concentrations were increased in both serum and EBC from patients with exacerbated COPD, when compared with patients with asthma attacks.

Neutrophils are considered to play a key role in the pathogenesis of COPD and they are increased in the airways of smokers and patients with COPD.[14,15] Exacerbation of COPD is thought to be associated with further amplification of inflammatory responses in the airways. Increased neutrophil influx and concomitant IL-8 elevation were observed in bronchoalveolar lavage (BAL) fluid of patients with exacerbated COPD.[16] In addition to leukotriene B4, IL-8 is another important neutrophil chemoattractant in COPD.[17] A study by Keatings et al,[8] showed significant increases in neutrophils and IL-8 in induced sputum from COPD patients compared with healthy individuals and asthmatic patients. In our study, EBC IL-8 concentration was higher in patients with exacerbated COPD than in patients with asthma attacks, suggesting that neutrophil-mediated airway inflammation is more dominant in COPD exacerbation than asthma attacks.

A previous study showed that the sputum IL-8 concentration in COPD patients rose from 69.8 ± 26.1 ng/mL at baseline to 127.3 ± 46.2 ng/mL at the time of exacerbation.[18] Another study enrolled 25 COPD patients and showed that the mean concentration of IL-8 in BAL fluid was 0.104 ng/mL.[19] In our study, the mean EBC IL-8 concentration in patients with exacerbated COPD was 5.27 pg/mL. Therefore, the amount of cytokines in EBC is much lower than that in BAL fluids or induced sputum. It was hypothesized that the aerosolized protein from the airway lining fluid was trapped in EBC during the process of condensation of water vapor in exhaled air. However, the mechanisms and site of EBC particle formation are not clearly understood.[13] A study showed that there is no significant correlation between inflammatory mediator concentration in the soluble phase of sputum and EBC.[6] Jackson et al[20] also found that biomarkers in EBC and BAL did not correlate with each other. Therefore, it is difficult to compare cytokine levels between EBC and induced sputum or BAL fluids. In a study by Ko et al, EBC IL-8 concentration measured by an ELISA kit was undetectable in most patients with exacerbated COPD.[21] The authors suggest that they might miss the peak of IL-8 at the start of COPD exacerbations, as the EBC collection was after 5 days of steroids treatment. In our study, the time of EBC collection in COPD patients was on Day 1 or Day 2 after hospitalization. The hypothesis of fluctuating cytokine levels over time may explain the

Table 2 Correlations of individual cytokine concentrations between serum and exhaled breath condensate in subgroup of patients.

Cytokine	Correlation of serum and EBC	
	COPD	Asthma
IL-8	$R = 0.085$, $p = 0.828$	$R = 0.54$, $p = 0.07$
IL-17	$R = 0.305$, $p = 0.425$	$R = -0.09$, $p = 0.793$
IL-4		$R = 0.114$, $p = 0.753$
TNF-α	$R = 0.06$, $p = 0.879$	

COPD = chronic obstructive pulmonary disease; IL-8 = interleukin-8; IL-17 = interleukin-17; IL-4 = interleukin-4; TNF-α = tumor necrosis factor-α.

difference in EBC IL-8 detection between our study and the previous study.

Tobacco smoking has been demonstrated to have a considerable effect on H_2O_2, 8-isoprostane, nitrite and nitrotyrosine concentrations in EBC.[22–25] In our study, healthy participants were all non-smokers, and patients with exacerbated COPD were all smokers. In patients with asthma attacks, no difference was observed in the mean cytokine levels between smokers and non-smokers (data not presented). This finding is limited for its small size in the asthma group. By contrast, patients who were smokers were requested to refrain from smoking for at least 12 hours before EBC collection. Therefore, the influence of smoking on these cytokine concentrations (IL-8, IL-17, IL-4, and TNF-α) in EBC needs further study.

Our study also showed that patients with exacerbated COPD had higher serum IL-8 levels than patients with asthma attacks. A study by Hollander et al,[19] demonstrated no significant difference in serum IL-8 concentrations between COPD and asthma patients, although the mean value was higher in the COPD group. The COPD and asthma patients in our study had acute exacerbations of their symptoms, which was different from the stable patients in the previous study. The amplified inflammation in COPD exacerbation may explain why we observed a significant difference in serum IL-8 between asthma and COPD patients. The inflammation in COPD patients appeared to extend beyond the lungs and this may play a role in the systemic effects of the disease and its associated comorbidities.[26] Patients with COPD were shown to have increased levels of circulating inflammatory markers, such as C-reactive protein or TNF-α.[27] In our study, an increased serum IL-8 level in patients with exacerbated COPD suggests a systemic inflammatory effect in these patients. However, the regulatory mechanisms of serum IL-8 expression are not clear. It is unknown whether systemic inflammation is a primary or secondary phenomenon related to pulmonary inflammation.

The accumulation of neutrophils in airways appears to be linked to the long-term course and exacerbation of COPD. Some studies demonstrated that stimulation of human airway epithelial cells with IL-17 *in vitro* causes the release of IL-8.[28–30] There is also evidence that pretreatment of the IL-17 protein with a specific neutralizing antibody attenuates its effect on IL-8.[30] IL-17 appears to recruit neutrophils via release of chemokines, such as IL-8, from epithelial cells. Although some patients with asthma were demonstrated to have an increased IL-17 concentration in their BAL fluids or EBC,[31,32] there is no information on the expression of IL-17 in COPD. In this study, there was no significant difference in EBC IL-17 concentrations among patients with exacerbated COPD, patients with asthma attacks and healthy individuals. This suggests that IL-17 may not play a causal role in IL-8 induction and the subsequent mobilization of neutrophils in patients with exacerbated COPD.

There are some limitations in this study. The relatively small sample size in each group makes it difficult to draw a definite and generalized conclusion. Also, age- and sex-matched control individuals were not enrolled for patients with exacerbated COPD and asthma attacks, respectively. Therefore, further studies are required to establish the influence of age and sex on EBC biomarkers. In addition, very low concentrations of cytokines, both in serum and EBC, were determined by the commercial ELISA kits. Most of the measured values fell below the validated detection limits of the relevant ELISAs, and were below the linear part of the sigmoid calibration curve. Since small variations in optical density on this portion of the curve can result in large changes in concentration derived by interpolation, it is possible that there was some error in the measurement of low cytokine concentrations. We found no significant correlation between serum and EBC cytokine levels in each subgroup of patients. This finding could be affected by the specific detection limit in the relevant ELISA. Further analytical methods are emerging to improve the sensitivity and validity of the measurement of biomarkers in EBC. The use of cytokine arrays, mass spectrometry, or nuclear magnetic resonance, should increase the sensitivity of the assays, and these methods may produce important new information in the future.[7]

In conclusion, this study demonstrated that both serum and EBC IL-8 concentrations were significantly higher in patients with exacerbated COPD than in patients with asthma attacks. These results provide evidence that IL-8 might play a more important role in airway and systemic inflammation in COPD exacerbations than in asthma attacks.

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Liu HC, Lu MC, Jan MS and Chen CM designed the research coordinately. Liu HC, Lin YC, Wu TC, and Hsu JY performed the research. Liu HC and Lin YC analyzed the data. Liu HC and Lu MC wrote the paper and Jan MS and Chen CM revised the paper.

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