

ORIGINAL ARTICLE

Elevated Serum Myeloid-Related Protein (MRP) 8/14 in Patients with Acute Exacerbation of Chronic Obstructive Pulmonary Disease

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SUMMARY

Background: The current study aims to evaluate the expression and clinical significance of myeloid-related protein (MRP) 8/14 in patients with acute exacerbation of chronic obstructive pulmonary disease (AECOPD).

Methods: The levels of MRP8/14, TNF- α , and IL-1 β in the serum of the patients with AECOPD were determined using ELISA assay. The correlation between the expression of MRP8/14 and TNF- α , IL-1 β , forced expiratory volume in one second FEV1 % pred in AECOPD patients was analyzed using Pearson's correlation assay. Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic value of serum MRP8/14 in AECOPD patients.

Results: The levels of MRP8/14, TNF- α , and IL-1 β in the serum of the patients with AECOPD were significantly higher than those in the control group. Furthermore, the expression of MRP8/14 was positively correlated with TNF- α , IL-1 β , and negatively correlated with FEV1 % pred. In addition, the level of serum MRP8/14 in GOLD 3-4 patients was higher than that in GOLD 1-2 patients. Meanwhile, the level of serum MRP8/14 in AECOPD patients with mMRC 3-4 was higher than that in patients with mMRC 0-2. ROC analysis showed that serum MRP8/14 could differentiate AECOPD patients from healthy controls.

Conclusions: Altogether, elevated serum MRP8/14 level plays a key role in chronic airway inflammation and may be a useful marker in the diagnosis of AECOPD patients.

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KEY WORDS

myeloid-related protein 8/14, acute exacerbation of chronic obstructive pulmonary disease, serum, chronic airway inflammation

INTRODUCTION

Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) refers to the sudden exacerbation of cough, expectoration, wheezing, and other symptoms caused by infection or other factors in COPD patients [1]. Severe AECOPD patients are often associated with acute respiratory failure, which leads to an increased risk of death [2]. However, there is a lack of effective indicators to predict the occurrence of AECOPD [3]. In AECOPD patients, inflammatory cells are activated and release a variety of cytokines and inflammatory media-

tors, including leukotriene B4, IL-8, TNF- α , and other mediators [4,5]. These mediators can result in the destruction of alveolar structure and promote the inflammatory response of neutrophils, but the mechanism is not fully understood [6].

Myeloid-related protein (MRP) 8/14 is a member of the calcium-binding S100 protein family and mainly expressed in myeloid cell lineage, such as neutrophils, monocytes and macrophages, to execute cytokine-like activities [7]. Previous studies have shown that several factors, including lipopolysaccharide (LPS), double-stranded RNA, IFN-g and TNF, induce the expression of MRP8/14 in response to bacterial challenge, such as *Escherichia coli*, and *Pseudomonas aeruginosa* [8-10]. MRP8/14 is involved in multiple biological processes, including cell survival and death, immune and inflammatory responses [11,12]. However, whether MRP8/14 participates in the progression of COPD has not been revealed.

In this study, the expression and clinical significance of MRP8/14 in serum of patients with AECOPD and healthy controls was evaluated, which may be helpful for improving the diagnosis of AECOPD.

MATERIALS AND METHODS

Patient samples

This study has been approved by the ethics committee of Beijing Luhe Hospital, and informed consent was signed by each subject. From June 2018 to February 2019, the serum of 43 patients with AECOPD was collected from Beijing Luhe Hospital and the serum of 20 healthy people as the control group. All AECOPD patients were confirmed by history, physical examination, chest X-ray and pulmonary function examination, and the diagnosis was in accordance with the Global Initiative for Chronic Obstructive Lung Disease. Details were shown in Table 1. The classification criteria of severity of airflow restriction in COPD were as follows [13]:

Gold level 1 (mild): FEV1 % pred \geq 80%;
 Gold Level 2 (moderate): 50% \leq FEV1 % pred < 80%;
 Gold Level 3 (severe): 30% \leq FEV1 % pred < 50%;
 Gold 4 (very severe): FEV1 % pred < 30%.

Enzyme-linked immunosorbent assay (ELISA)

Venous blood (3 - 5 mL) was collected from all subjects from the elbow vein in the morning when they were fasting on the day of pulmonary function examination. The blood was centrifuged at 4°C for 10 minutes. After centrifugation, the serum was collected and placed at -80°C until detection. The level of serum MRP8/14 was detected using a LEGEND MAX™ Human MRP8/14 (Calprotectin) ELISA Kit (Biolegend, USA) according to the instructions.

Statistical analysis

The data were represented as the mean \pm standard deviation (SD). The two-tailed unpaired Student's *t*-test was

used for comparisons of two groups. The one-way ANOVA multiple comparison test (SPSS 20.0) followed by Tukey's post hoc test were used for comparisons of two more groups. Receiver operating characteristic (ROC) curves were used to assess MRP8/14 as a biomarker, and the area under the curve (AUC) was reported (version 20.0, IBM SPSS Statistics for Windows; IBM Corp, Armonk, NY, USA). $p < 0.05$ was considered significant.

RESULTS

Comparison of general data between AECOPD patients and healthy controls

There was no difference in gender, age, height, and hemoglobin between AECOPD patients and healthy controls. Body mass index (BMI), forced expiratory volume in one second (FEV1), FEV1 prediction (pred) and albumin levels of AECOPD patients were significantly lower than those of the healthy control group (Table 1). Smoking index, leukocyte, neutrophil and high-sensitivity C-reactive protein (hs-CRP) were significantly higher in AECOPD patients than those of the healthy control group, as shown in Table 1.

Elevated levels of serum MRP8/14, TNF- α , and IL-1 β in AECOPD patients

First, we compared the levels of MRP8/14, TNF- α , and IL-1 β in AECOPD patients and healthy controls. As shown in Figure 1, the levels of MRP8/14, TNF- α , and IL-1 β in the serum of the patients with AECOPD were significantly higher than those in the control group (Figure 1A, 1B, and 1C).

The correlation between the expression of MRP8/14 and TNF- α , IL-1 β , and FEV1 % pred in AECOPD patients

Previous studies have indicated the key roles of inflammatory response during the progression of AECOPD [14,15]. Hence, we evaluated the correlation between serum MRP8/14 and TNF- α and IL-1 β . Our data showed that the expression of MRP8/14 was positively correlated with TNF- α ($r = 0.583$, $p < 0.01$), IL-1 β ($r = 0.686$, $p < 0.001$), and negatively correlated with FEV1% pred ($r = -0.699$, $p < 0.01$) (Figure 2A, 2B and 2C).

Relationship between clinical characteristics and serum MRP8/14 levels in patients with AECOPD

We then analyzed the level of serum MRP8/14 according to the classification of lung function. As shown in Figure 3A, the level of serum MRP8/14 in GOLD 3 - 4 patients was higher than that in GOLD 1 - 2 patients. Furthermore, the level of serum MRP8/14 in AECOPD patients with mMRC 3 - 4 was higher than that in AECOPD patients with mMRC 0 - 2 (Figure 3B). There was no significant correlation between serum MRP8/14 and gender or smoking.

Table 1. Comparison of general data between AECOPD patients and healthy controls.

	Healthy controls	AECOPD patients	p-value
No.	20	43	
M/F	15/5	39/4	0.128
Age	68.55 ± 9.01	71.53 ± 7.53	0.174
Height (cm)	161 ± 5.62	162 ± 6.31	0.351
BMI	23.57 ± 3.97	20.77 ± 3.53	0.006
Smoking (cigarettes/year)	90 ± 65	736 ± 473	< 0.001
FEV1 (L)	2.42 ± 0.41	1.01 ± 0.40	< 0.001
FEV1 % pred	102.49 ± 12.08	42.22 ± 17.31	< 0.001
Leukocyte count (x 10 ⁹)	6.16 ± 1.28	9.38 ± 3.81	< 0.001
Neutrophil count (x 10 ⁹)	3.88 ± 0.86	6.67 ± 3.38	< 0.001
Hemoglobin	135 ± 13.56	132 ± 14.53	0.404
Albumin	40.86 ± 2.00	36.25 ± 3.73	< 0.001
hs-CRP	2.88 ± 1.31	37.28 ± 15.87	< 0.001

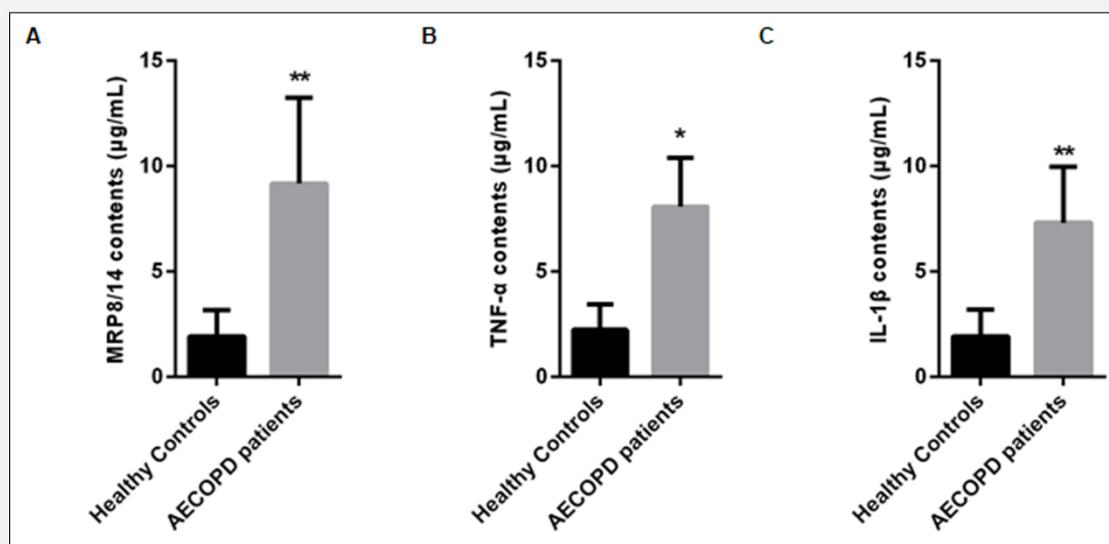


Figure 1. ELISA assay was performed to analyze the levels of MRP8/14, TNF- α , and IL-1 β in serum between AECOPD and healthy controls.

The levels of MRP8/14 (A), TNF- α (B), and IL-1 β (C) in the serum of the patients with AECOPD were significantly higher than those in the control group. * $p < 0.05$, ** $p < 0.01$ vs. controls.

Diagnostic value of serum MRP8/14 in AECOPD patients

ROC analysis was carried out to evaluate the diagnostic value of serum MRP8/14 in AECOPD patients. The area under curve of serum MRP8/14 was 0.937. The

sensitivity and specificity of serum MRP8/14 in the diagnosis of AECOPD were 90% and 100%, respectively, when the cutoff value was 4.87 $\mu\text{g/mL}$. These data indicated that serum MRP8/14 could differentiate AECOPD patients from healthy controls.

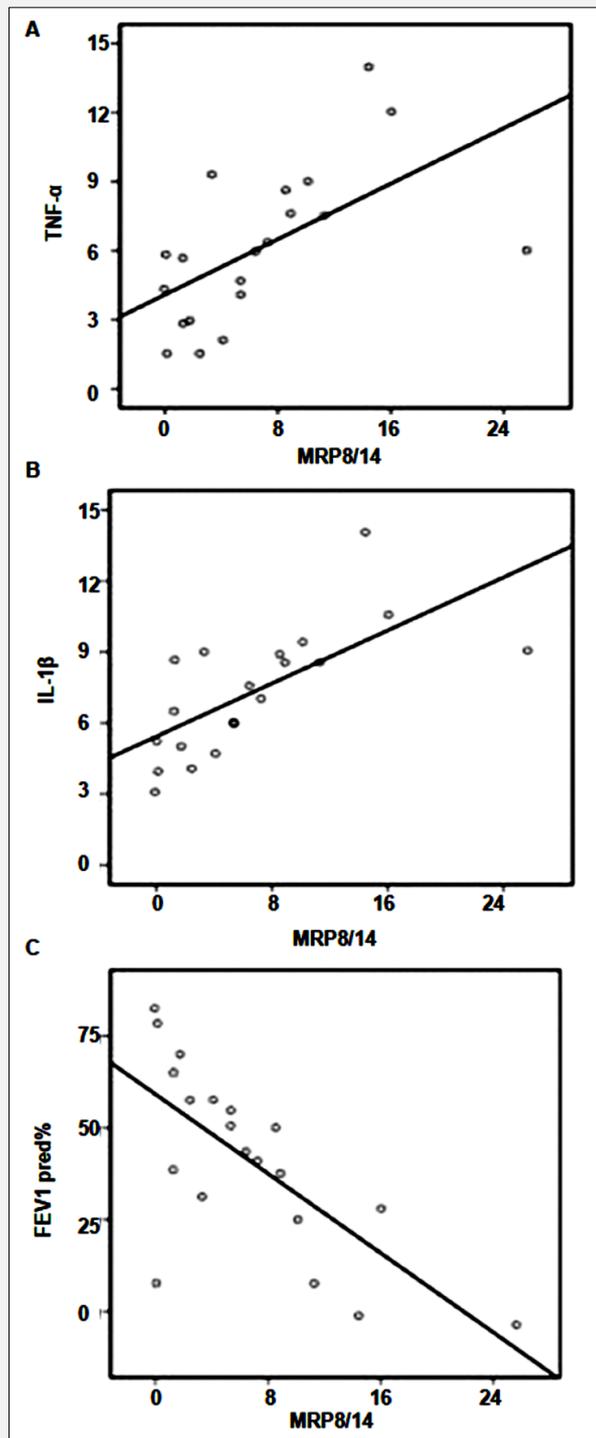


Figure 2. Pearson's correlation assay was performed to analyze the correlation between the expression of MRP8/14 and TNF- α , IL-1 β , and FEV1 % pred in AECOPD patients.

Serum MRP8/14 was positively correlated with TNF- α (A), IL-1 β (B), and negatively correlated with FEV1 % pred (C).

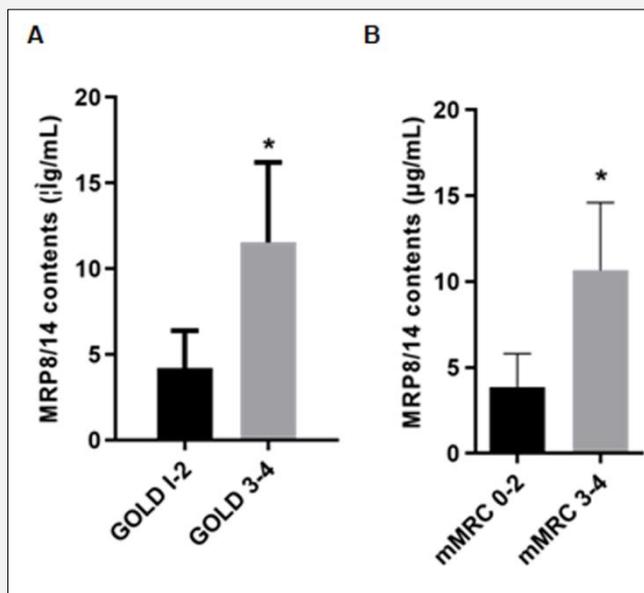


Figure 3. The level of serum MRP8/14 was determined according to the clinical characteristics.

(A) The level of serum MRP8/14 in GOLD 3 - 4 patients was higher than that in GOLD 1 - 2 patients. (B) The level of serum MRP8/14 in AECOPD patients with mMRC 3 - 4 was higher than that in AECOPD patients with mMRC 0 - 2. * $p < 0.05$ vs. controls.

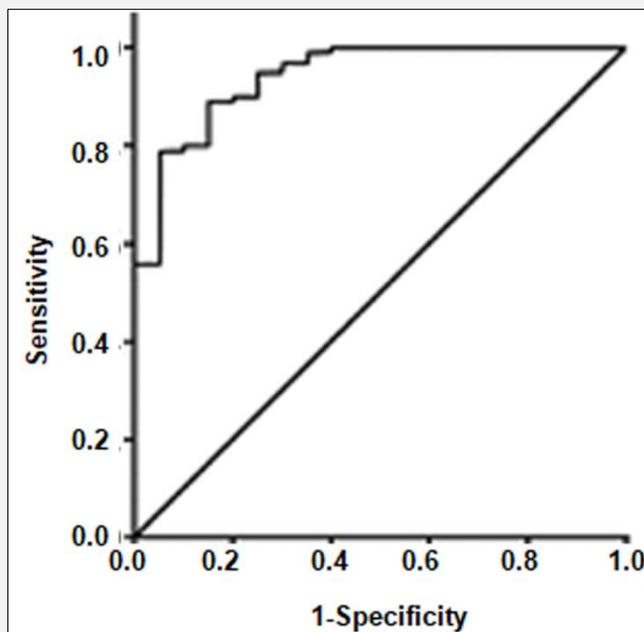


Figure 4. ROC analysis was performed to evaluate the diagnostic value of serum MRP8/14 in AECOPD patients.

DISCUSSION

Inflammatory cells were activated in patients with AECOPD [16,17]. From the results of this study, the level of leukocytes, neutrophils, hs-CRP, and other inflammatory markers in the blood of patients with AECOPD was significantly higher than that of the healthy group, indicating that there was inflammatory response in patients with AECOPD. It is reported that the expression of MRP8/14 is induced in monocytes or macrophages via different inflammatory stimulators, such as LPS and TNF [11,12,18]. MRP8/14 is considered to be involved in the development of various inflammatory diseases [10,12,19], but its role in COPD has not been reported.

In this study, the expression of MRP8/14 in serum of AECOPD group was significantly higher than that of the control group, and the expression of TNF- α and IL-1 β was also increased. Furthermore, in the AECOPD group, the level of MRP8/14 was positively correlated with TNF- α and IL-1 β , which also verified the positive feedback loop of MRP8/14, TNF- α , and IL-1 β in the inflammatory factor network. A previous study has suggested that MRP8/14/TLR4/NF- κ B/TNF- α activated by platelet-neutrophil interaction is a key inflammatory signaling pathway for atherosclerosis (AS) progression [20]. We propose that MRP8/14 is involved in the airway inflammatory response of AECOPD and may be a new therapeutic target.

In addition, this study also found that the expression of MRP8/14 in serum of patients with AECOPD was negatively correlated with the predicted value of FEV1 % pred, suggesting that the level of MRP8/14 was correlated with the severity of airflow restriction. Therefore, in addition to the above-mentioned involvement in immune and inflammatory response functions, MRP8/14 may also participate in the proliferation of airway smooth muscle and airway remodeling, leading to further deterioration of ventilation function. But further study is necessary in the future.

In addition, the sensitivity and specificity of MRP8/14 in the diagnosis of AECOPD were 90% and 100%, respectively, indicating that serum MRP8/14 can effectively distinguish AECOPD patients from healthy controls. However, there are limitations in the present study. First, the total number of samples in this study is small, and we still need to expand the sample size. Second, in order to provide more theoretical support and guidance for the diagnosis, treatment, and prevention of AECOPD, we should continue to increase the groups of stable COPD and severe smokers, and perform comprehensive research combining with factors such as race, environment, and phenotype differences.

Altogether, MRP8/14, an important inflammatory mediator, plays a key role in the progression of chronic airway inflammation in AECOPD patients. In the future, the specific regulatory mechanism of MRP8/14 in airway inflammation of AECOPD and the intervention measures targeting MRP8/14 need further study.

Declaration of Interest:

The authors declare no competing financial interests.

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