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Elevated serum levels of TNF- α , IL-8, and ECP can be involved in the development and progression of bronchial asthma

Running title: TNF- α , IL-8 and ECP and bronchial asthma

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Abstract

Objective: This study aimed to explore the value of elevated serum levels of tumor necrosis factor- α (TNF- α), interleukin-8 (IL-8) and eosinophil cationic protein (ECP) in the diagnosis of bronchial asthma (BA).

Methods: Totally 170 patients with BA (case group, 85 patients in acute attack and 85 patients in clinical remission) and 150 healthy individuals (control group). Enzyme-linked immunosorbent assay (ELISA) and receiver operating characteristic (ROC) curves were calculated for the contents and diagnostic values of serum TNF- α , IL-8, and ECP in BA.

Results: Compared with the control group, the TNF- α , IL-8, and ECP levels were higher in patients in acute attack and clinical remission ($P < 0.05$). The serum level of TNF- α was positively correlated with IL-8 and ECP ($P < 0.05$). ROC curves showed that the diagnostic threshold value of IL-8 was 13.53 ng/ml, its area under the curve (AUC) was 0.87, its specificity was 99.3%, and its sensitivity was 57.6%. The diagnostic threshold value of TNF- α was 1.29 ng/ml with AUC was 0.94, specificity was 89.3% and sensitivity was 83.5%. ECP showed 7.22 ng/ml diagnostic threshold value (AUC = 0.88, specificity = 74.0%, sensitivity = 86.5%). The $FEV_1/pre(\%)$ and FEV_1/FVC were negatively correlated and the $Z5/pre(\%)$ and resonance frequency (Fres) were positively correlated with the serum levels of TNF- α , IL-8, and ECP in patients in acute attack and in clinical remission (all $P < 0.05$).

Conclusion: Our findings reveal that elevated serum levels of TNF- α , IL-8 and ECP can be involved in the development and progression of BA.

Keywords

Bronchial asthma, Tumor necrosis factor- α , Interleukin-8, Eosinophil cationic protein

Introduction

Bronchial asthma is characterized by clinical symptoms, chronic airway inflammation, and exaggerated bronchoconstriction when exposed to allergens and other factors. Its asthma-related symptoms are coughing, wheezing, chest tightness, expectoration, and dyspnea, typically expiratory dyspnea with episodic wheezing [1, 2]. According to data from the global initiative for asthma (GINA), the global prevalence of bronchial asthma ranges from 1% to 21% in adults, and more than 20% of 6 to 7-year old children suffer from this disease [3]. In modern medicine, TH1/TH2 imbalance, cytokines, matrix metalloproteinase (MMP), tissue inhibitor of metalloproteinase (TIMP), genetic factors, and neural regulation play important roles in the occurrence and development of asthma [4-6].

Tumor necrosis factor- α (TNF- α), an important cytokine in the innate immune response, directly kills tumor cells but has no toxic effect on normal cells, which incites an inflammatory response [7]. Previous experiments have found that TNF- α plays a critical regulatory function in activating the airway inflammation associated with asthma [8]. Very similar to TNF- α , interleukin-8 (IL-8) is an anti-inflammatory cytokine which has a regulatory effect on the airway inflammation caused by asthma [9]. Eosinophil cationic protein (ECP), secreted by activated eosinophils, is a specific marker of eosinophils [10]. It has been found that activated eosinophils and serum ECP levels play important roles in the pathogenesis of bronchial asthma [11-13]. Recently, several studies have revealed that TNF- α , IL-8, and ECP have strong regulatory effects on asthma. This was demonstrated by their relatively high sputum levels [14-16]. Therefore, this

study intends to explore the value of elevated serum levels of TNF- α , IL-8, and ECP in the diagnosis of bronchial asthma.

Materials and methods

Study subjects

A total of 170 patients with bronchial asthma (89 males and 81 females, aged from 22 to 55 years old, with a mean age of 35.56 ± 7.86 years) admitted into our hospital between May 2013 to July 2014 were enrolled in this study. Included were 85 patients with bronchial asthma in acute attack (bronchial asthma occurred suddenly or the original symptoms exacerbated) and 85 patients with bronchial asthma in clinical remission (disappearance of the symptoms and signs after the treatment or without treatment, lung function recovered to the level before acute attack and kept a stable state for no less than 3 months), according to the diagnostic criteria of bronchial asthma (GINA2011) on the basis of their medical history. Their clinical symptoms were mainly paroxysmal chest tightness, wheezing, shortness of breath, cough, and dyspnea, all of which were reactions to bronchial hyper responsiveness or airway obstruction. The positive rate of bronchial provocation test (BPT) after the inhalation of methacholine was $PD_{20} < 2.505$ mg, the positive rate of bronchial dilation test (BDT) after the inhalation of salbutamol was $FEV_1\% \geq 12\%$ and an increase of 200 ml). The inclusion criteria were as follows: 1) patients did not accept systemic hormones, immune globulin, or immunosuppressive agents for 3 months; 2) patients did not associate with other autoimmune diseases; 3) patients did not develop chronic obstructive pulmonary disease (COPD), phthisis, lung cancer, or other lung diseases; 4) patients did not develop infectious diseases, used antibiotics but did not use adrenocortical hormones or other

immunomodulators recently. The exclusion criteria were as follows: 1) patients had COPD, pulmonary infection, or interstitial pneumonia; 2) allergic habits of patients were not included; 3) patients suffered from hepatitis, various diseases of the immune system, acute and chronic leukemia, or thymoma; 4) patients underwent organ transplantation, also pregnant women and lactating women were excluded. A total of 150 healthy individuals were selected as the control group, including 78 males and 72 females (aged from 20 to 54 years; mean age 36.71 ± 8.17 years). There were no statistical differences in age or gender between the groups. This study was approved by the Ethics Committee of our hospital, and all patients signed the written informed consent.

Blood specimen treatment

Venous blood specimens (5ml) were taken from subjects' right upper extremity and mixed with anticoagulant sodium citrate solution, followed by a 10-min centrifugation at 450 rpm (Avanti J-26X, Beckman, USA) after standing for 30-min. The supernatant was extracted and kept at -70°C (DW-86L388A, Haier, China) for cytokine analysis.

Detection of serum TNF- α , IL-8 and ECP levels

Serum was extracted from peripheral venous blood collected from patients with bronchial asthma both in acute attack and in clinical remission as soon as they were admitted to the hospital to detect TNF- α , IL-8, and ECP levels in serum via enzyme-linked immunosorbent assay (ELISA). IL-8 and TNF- α kits were produced by Anogen-Yes Biotech Laboratories Ltd., Ontario, Canada. The ELISA kit for ECP, TNF- α , IL-8, and ECP standards was purchased from Pharmacia Company, Sweden. The changes in TNF- α , IL-8, and ECP levels in serum were

measured in hospitalized patients one day after the serum was isolated. All detections were strictly in accordance with kit instructions.

Pulmonary function examination

The PFT multifunctional pulmonary function detector (JEGER Company, Würzburg, Germany) was used to detect the percentage of predicted value of forced expiratory volume at 1st second ($FEV_{1/pre}(\%)$) and forced expiratory volume at 1st second (FEV_1) vs. forced vital capacity (FVC) ($FEV_1/FVC(\%)$). The impulse oscillometry (IOS) pulmonary function detector (JEGER Co. Germany) was used to detect total respiratory impedance (Z5), airway viscous resistance at 5 Hz (R5), airway viscous resistance at 20 Hz (R20), electrical impedance at 5 Hz (X5), resonance frequency (Fres), central airway resistance (Rc), and peripheral airway resistance (RP). The instrument error range is < 3% and its accuracy is to 0.05 L. Additionally, each test was repeated three times.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (SPSS Inc, Chicago, IL, USA). Expressed as ratio or percentage, enumeration data among groups were compared by Chi-square test. Measurement data were expressed as mean $\pm$ standard deviation (SD), and the *t*-test was used for the comparison between the two groups, while one-way variance was performed to analyze the comparison among several groups. The correlation between each index was analyzed by the Pearson correlation method. A receiver operating characteristic (ROC) curve was adopted to evaluate the diagnostic values of TNF- α , IL-8, and ECP for patients with bronchial asthma in

acute attack and in clinical remission. All statistical tests were two-tailed probability tests, and $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics between the case group and the control group [17]

A total of 170 patients with bronchial asthma (89 males and 81 females, aged from 22 to 55 years old, with mean age of 35.56 ± 7.86 years) were selected for this study, including 85 patients with bronchial asthma in acute attack and 85 patients with bronchial asthma in clinical remission. There were 93 patients with mild bronchial asthma, 46 patients with moderate bronchial asthma, and 31 patients with severe bronchial asthma. Meanwhile, 150 healthy individuals were recruited as the control group, consisting of 78 males and 72 females (aged from 20 to 54 years; mean age 36.71 ± 8.17 years). There was no statistical significance in gender, age, obesity, smoking history, or drinking history between the case group and the control group (all $P > 0.05$) (Table 1).

Serum levels of TNF- α , ECP, and IL-8 in the control group and the case group

The contents of serum TNF- α , ECP, and IL-8 in the case group and control group are shown in Table 2. Serum TNF- α , ECP, and IL-8 levels in the case group of acute attack were all significantly higher than those in the control group and the case group of clinical remission (all $P < 0.01$).

Correlation analysis of serum levels of TNF- α , ECP and IL-8 of patients with bronchial asthma in acute attack and clinical remission

In patients with bronchial asthma in acute attack, TNF - α levels in serum was positively correlated with IL-8 ($r = 0.647$, $P < 0.01$, 95%CI = 0.503 ~ 0.756) and ECP levels ($r = 0.764$, $P < 0.01$, 95%CI = 0.658 ~ 0.840), and the IL-8 level was positively correlated with the ECP level ($r = 0.716$, $P < 0.01$, 95%CI = 0.593 ~ 0.806). In addition, in the case group of clinical remission, the TNF - α level in serum bore a positive correlation with IL-8 ($r = 0.480$, $P < 0.01$, 95%CI = 0.297 ~ 0.629) and ECP levels ($r = 0.524$, $P < 0.01$, 95%CI = 0.350 ~ 0.663), and the IL-8 level displayed a positive correlation with the ECP level ($r = 0.532$, $P < 0.01$, 95%CI = 0.359 ~ 0.669) (Figure 1).

ROC curves of the serum levels of TNF- α , IL-8 and ECP in diagnosis of asthma

The ROC curves of serum TNF- α , IL-8, and ECP levels in the diagnosis of asthma are presented in Figure 2. For IL-8, the diagnostic threshold value was 13.53 ng/ml with an area under the curve (AUC) of 0.87, a specificity of 99.3%, and a sensitivity of 57.6% (95%CI = 0.830 ~ 0.944). For TNF- α , the diagnostic threshold value was 1.29 ng/ml with an AUC of 0.94, a specificity of 89.3%, and a sensitivity of 83.5% (95%CI = 0.903 ~ 0.980). The diagnostic threshold value of ECP was 7.22 ng/ml with an AUC of 0.88, a specificity of 74.0%, and a sensitivity of 86.5% (95%CI = 0.833 ~ 0.947).

Correlation analysis between the serum levels of TNF- α , IL-8 and ECP and pulmonary function of patients

The comparison of pulmonary function parameters in each group is shown in Table 3. When compared with the control group, there was a decrease in $FEV_1/pre(\%)$ and FEV_1/FVC , but an increase in Z5, R5, R20, Fres, and Rc in the case group of acute attack (all $P < 0.05$). In the case group of clinical remission, the differences in $FEV_1/pre(\%)$, FEV_1/FVC , and X5 when compared with the control group was not statistically significant, while $Z5/pre(\%)$, $R5/pre(\%)$, $R20/pre(\%)$, Rc ($kPa.L^{-1}.S^{-1}$), and RP ($kPa.L^{-1}.S^{-1}$) were all increased (all $P < 0.05$).

Correlation analysis was performed between four relatively correlated indexes ($FEV_1/pre(\%)$, FEV_1/FVC , $Z5/pre(\%)$, and Fres) and TNF- α , IL-8, and ECP (Table 4). In the case group of acute attack, $FEV_1/pre(\%)$ was negatively associated with TNF- α ($r = -0.54$, 95%CI = $-0.679 \sim -0.375$, $P < 0.001$), ECP ($r = -0.47$, 95%CI = $-0.623 \sim -0.289$, $P < 0.001$) and IL-8 levels ($r = -0.39$, 95%CI = $-0.556 \sim -0.192$, $P < 0.001$) at serum levels. FEV_1/FVC also shared a negative correlation with serum TNF- α ($r = -0.49$, 95%CI = $-0.635 \sim -0.307$, $P < 0.001$), ECP ($r = -0.53$, 95%CI = $-0.666 \sim -0.355$, $P < 0.001$) and IL-8 levels ($r = -0.38$, 95%CI = $-0.546 \sim -0.178$, $P < 0.001$). $Z5/pre(\%)$ was positively correlated with serum TNF- α ($r = 0.62$, 95%CI = $0.465 \sim 0.734$, $P < 0.001$), ECP ($r = 0.61$, 95%CI = $0.451 \sim 0.725$, $P < 0.001$) and IL-8 levels ($r = 0.51$, 95%CI = $0.334 \sim 0.653$, $P < 0.001$). Fres was positively associated with serum TNF- α ($r = 0.50$, 95%CI = $0.321 \sim 0.644$, $P < 0.001$), ECP ($r = 0.66$, 95%CI = $0.523 \sim 0.767$, $P < 0.001$) and IL-8 levels ($r = 0.52$, 95%CI = $0.341 \sim 0.657$, $P < 0.001$). In the case group of clinical remission, there was a negative correlation between $FEV_1/pre(\%)$ and serum TNF- α ($r = -0.34$, 95%CI =

-0.517 ~ -0.138, $P = 0.001$), ECP ($r = -0.56$, 95%CI = -0.689 ~ -0.391, $P < 0.001$) and IL-8 levels ($r = -0.71$, 95%CI = -0.805 ~ -0.590). FEV_1/FVC was negatively associated with serum TNF- α ($r = -0.86$, 95%CI = -0.909 ~ -0.796, $P < 0.001$), ECP ($r = -0.58$, 95%CI = -0.708 ~ -0.423, $P < 0.001$) and IL-8 levels ($r = -0.48$, 95%CI = -0.631 ~ -0.301, $P < 0.001$) as well. $Z5/pre(\%)$ showed a positive correlation with serum TNF- α ($r = 0.82$, 95%CI = 0.730 ~ 0.877, $P < 0.001$), ECP ($r = 0.57$, 95%CI = 0.405 ~ 0.697, $P < 0.001$) and IL-8 levels ($r = 0.44$, 95%CI = 0.248 ~ 0.595, $P < 0.001$). $Fres$ was positively correlated with serum TNF- α ($r = 0.52$, 95%CI = 0.344 ~ 0.660, $P < 0.001$), ECP ($r = 0.57$, 95%CI = 0.404 ~ 0.697, $P < 0.001$) and IL-8 levels ($r = 0.55$, 95%CI = 0.378 ~ 0.681, $P < 0.001$).

Discussion

Asthma is a complex and chronic inflammatory disease involving the critical interaction of several important cytokines, such as inflammatory cells and inflammatory media [18]. When bronchial asthma is in acute attack, it arouses airway hyper-responsiveness and releases cytokines which lead to bronchial spasms and airflow obstruction [19]. During this process, leukocytes play an important role by activating and releasing a large amount of inflammatory medium which can trigger or exaggerate an inflammatory reaction to enhance the inflammatory response and exacerbate disease progression [20, 21]. Therefore, because the serum levels of TNF- α , IL-8, and ECP correlated with the development and progression of bronchial asthma, studying the relationship between these factors and bronchial asthma can be used to guide the treatment of bronchial asthma. Also, this study indicated that serum levels of TNF- α , IL-8, and ECP can be possible markers in distinguishing the severity of bronchial asthma.

As shown in the results, the contents of serum TNF- α , IL-8, and ECP levels in asthma patients were far higher than those in normal individuals. Serum TNF- α , IL-8, and ECP levels can be correlated with the development and progression of asthma, so their levels in the case group of acute attack were much higher than in the case group of clinical remission. An asthma attack is associated with the activation of leukocytes. Gleaning a better understanding of the functions, reciprocal regulation, and counterbalance of subsets of immune and inflammatory cells that interact through interleukins, interferons, TNF- α , and TGF- β offers opportunities for immune interventions and novel treatment modalities in the era of the development of biological immune response modifiers, particularly targeting these molecules or their receptors [22]. TNF- α has important biological effects on airway inflammation and remodeling, and can promote airway epithelium injury and airway hyper-responsiveness [18, 23]. Also, TNF- α in its soluble form can stimulate the induction of inflammatory cytokines, including IL-1 β , IL-6, and IL-8 [7]. Therefore, TNF- α has been postulated to be a critical mediator contributing to airway inflammation [24]. In the stage of acute attack, TNF- α principally promotes inflammation and decreases the pulmonary ventilation function [25]. We evaluated the effects of IL-17A, IL-17E, and IL-17F in combination with other stimuli such as TNF- α on the production and expression of IL-8 in human bronchial epithelial cells [26]. As an important up-regulated cytokine for airway inflammation, IL-8 is mainly secreted during asthma in acute attack and during an inflammatory reaction in clinical remission [27]. IL-8 plays an important role in respiratory disease and is a known regulator of pulmonary inflammation and immunity [28]. IL-8 is secreted by epithelial cells. With chemotaxis and activation effects on neutrophils, IL-8 leads to neutrophil accumulation in the airways of patients with bronchial asthma. IL-8 directed neutrophils release

inflammatory media like leukotrienes and platelet activating factor, which directly results in airway spasms [29]. Furthermore, IL-8 can effectively regulate the expression of adhesion molecules to strengthen the adhesion of leukocytes and endothelial cells, promoting the release of ECP [30]. ECP is a protein secreted by eosinophils after being activated by antigens. It is cytotoxic and can disrupt the protective pulmonary epithelial barrier, inducing a further inflammatory response [31]. A previous study demonstrated that with an increased concentration of IL-8, the activity of eosinophils was further strengthened, which increased the amount of ECP released [27, 32]. Therefore, TNF- α , IL-8, and ECP levels were significantly increased during an asthma attack.

The results of this study revealed that in asthma patients, $Z5/pre(\%)$ and $Fres$ levels were greatly increased while $FEV_1/pre(\%)$ and FEV_1/FVC levels were largely decreased. This indicated that the airway has been interfered with, resulting in dyspnea and reduced ventilation volume. To some extent, these results were correlated with the expression levels of TNF- α , IL-8, and ECP. When asthma is in acute attack, with TNF- α increasing the concentration of IL-8 [25], IL-8 induces monocyte and T cells to adhere. This potentiates eosinophil recruitment and activation in serum to secrete ECP [30]. As a strong alkaline toxic protein, ECP has a strong effect on the airway epithelium and nasal mucosa epithelium, which is also involved in airway hyper-responsiveness [31, 33]. Reddy *et al.* reported that IL-8 played a vital role in airway inflammation via its involvement in chemotaxis and infiltration of airway neutrophils rather than eosinophils during acute asthma attacks [34]. Aside from a strong influence on chemotaxis and neutrophils, IL-8 can induce the degranulation and bactericidal effects of neutrophils. Meanwhile, activated neutrophils can secrete IL-8 and form a positive feedback loop, which

further aggravates airway inflammation [29]. Furthermore, the results of this study demonstrated that TNF- α , IL-8, and ECP functioned via activating an inflammatory reaction and promoting related pathological features. With their remarkable regulatory functions in asthma, TNF- α , IL-8, and ECP are expected to serve as biomarkers in the diagnosis of asthma.

Our study suggests that elevated serum levels of TNF- α , IL-8, and ECP can be involved in the development and progression of bronchial asthma, and provided some references for clinical diagnoses of bronchial asthma. Our findings can be clinically helpful in finding therapeutic strategies for bronchial asthma involving serum TNF- α , IL-8, and ECP levels. However, due to the limitations of sample size and differences in male to female ratios, we suggest that more prospective studies with larger sample sizes be conducted to provide more precise estimates to confirm that the serum levels of TNF- α , IL-8, and ECP are correlated with different severities of bronchial asthma. This will help us get closer to the goal of developing new genetic therapeutic strategies for the treatment of bronchial asthma.

Competing interests

None.

Acknowledgments

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Table 1. Baseline characteristics of study subjects between the case group and the control group

Characteristic	Case group	Control group	t/ χ^2	P
	(n = 170)	(n = 150)		
Gender (male/female)	89/81	78/72	0.004	0.950
Age (years)	35.56 $\pm$ 7.86	36.71 $\pm$ 8.17	1.282	0.201
Obesity (yes/no)	83/87	73/77	0.001	0.978
Smoking history (yes/no)	78/92	69/81	0.001	0.983
Drinking history (yes/no)	76/94	78/72	1.698	0.193
Treatment history (yes/no)	105/65	0/150	-	< 0.001

Table 2. Serum levels of TNF- α , ECP and IL-8 in the control and the case group

	Control group (n = 150)	Case group		95%CI ¹	95%CI ²	95%CI ³
		Acute attack (n = 85)	Clinical remission (n = 85)			
TNF- α (ng/ml)	0.91 $\pm$ 0.34	2.57 $\pm$ 0.4	1.37 $\pm$ 0.17	1.556 ~ 1.762	0.354 ~ 0.560	1.086 ~ 1.319
ECP (ng/ml)	5.74 $\pm$ 2.41	15.44 $\pm$ 6.99	8.65 $\pm$ 1.57	8.416 ~ 11.000	1.626 ~ 4.210	5.330 ~ 8.250
IL-8 (ng/ml)	7.81 $\pm$ 2.89	18.77 $\pm$ 3.34	10.84 $\pm$ 3.02	9.989 ~ 11.940	2.057 ~ 4.006	6.830 ~ 9.034

Note: 95%CI¹, comparison between the control group and the case group of acute attack;

95%CI², comparison between the control group and the case group of clinical remission;

95%CI³, comparison between the case group of acute attack and the case group of clinical

remission. CI, confidence interval; TNF, tumor necrosis factor; ECP, eosinophil cationic protein;

IL, interleukin.

Table 3. Comparison of pulmonary function parameters between the control and case groups

Group	Control group (n = 150)	Case group	
		Acute attack (n = 85)	Clinical remission (n = 85)
$FEV_1/pre(\%)$	99.17 ± 7.73	$74.28 \pm 4.98^*$	97.72 ± 8.46
$FEV_1/FVC(\%)$	84.09 ± 6.58	$70.70 \pm 6.20^*$	82.55 ± 5.36
$Z5/pre(\%)$	0.32 ± 0.10	$0.77 \pm 0.23^*$	$0.48 \pm 0.15^\#$
$R5/pre(\%)$	124.65 ± 23.86	$161.18 \pm 21.12^*$	$137.52 \pm 18.15^\#$
$R20/pre(\%)$	108.23 ± 10.83	$141.89 \pm 10.45^*$	$126.79 \pm 12.03^\#$
$X5 (kPa.L^{-1}.S^{-1})$	-0.29 ± 0.15	$-0.52 \pm 0.22^*$	-0.30 ± 0.18
$Fres (HZ)$	10.71 ± 2.09	$19.60 \pm 5.80^*$	$12.18 \pm 5.52^\#$
$Rc (kPa.L^{-1}.S^{-1})$	0.12 ± 0.04	$0.34 \pm 0.11^*$	$0.22 \pm 0.04^\#$
$Rp (kPa.L^{-1}.S^{-1})$	0.21 ± 0.04	$0.48 \pm 0.17^*$	$0.32 \pm 0.10^\#$

Note: FEV_1 : forced expiratory volume at 1st second; $FEV_1/pre(\%)$: the percentage of predicted value of forced expiratory volume at 1st second; FVC : forced vital capacity; $Z5$: total respiratory impedance; $R5/pre(\%)$: the percentage of predicted value of total respiratory impedance; $R5$: airway viscous resistance at 5 Hz; $R20$: airway viscous resistance at 20 Hz; $X5$: electrical impedance at 5 Hz; $Fres$: resonance frequency; Rc : central airway resistance; Rp : peripheral airway resistance; *, both $P < 0.05$ when the case group of acute attack was compared with the control group and the case group of clinical remission; #, $P < 0.05$ when the case group of clinical remission was compared with the control group.

Table 4. Correlation analysis between serum factors and pulmonary function parameters in the case group

	Acute attack (n = 85)			Clinical remission (n = 85)		
	TNF- α	ECP	IL-8	TNF- α	ECP	IL-8
<i>FEV₁/pre(%)</i>	-0.54 (-0.679 ~ -0.375)	-0.47 (-0.623 ~ -0.289)	-0.39 (-0.556 ~ -0.192)	-0.34 (-0.517 ~ -0.138)	-0.56 (-0.689 ~ -0.391)	-0.71 (-0.805 ~ -0.590)
<i>FEV₁/FVC(%)</i>	-0.49 (-0.635 ~ -0.307)	-0.53 (-0.666 ~ -0.355)	-0.38 (-0.546 ~ -0.178)	-0.86 (-0.909 ~ -0.796)	-0.58 (-0.708 ~ -0.423)	-0.48 (-0.631 ~ -0.301)
<i>Z5/pre(%)</i>	0.62 (0.465 ~ 0.734)	0.61 (0.451 ~ 0.725)	0.51 (0.334 ~ 0.653)	0.82 (0.730 ~ 0.877)	0.57 (0.405 ~ 0.697)	0.44 (0.248 ~ 0.595)
<i>Fres (HZ)</i>	0.50 (0.321 ~ 0.644)	0.66 (0.523 ~ 0.767)	0.52 (0.341 ~ 0.657)	0.52 (0.344 ~ 0.660)	0.57 (0.404 ~ 0.697)	0.55 (0.378 ~ 0.681)

Note: *FEV₁*, forced expiratory volume at 1st second; *FEV₁/pre(%)*: the percentage of predicted value of forced expiratory volume at 1st second; *FVC*, forced vital capacity; *Z5*, total respiratory impedance; *Fres*, resonance frequency; *TNF*, tumor necrosis factor; *ECP*, eosinophil cationic protein; *IL*, interleukin.

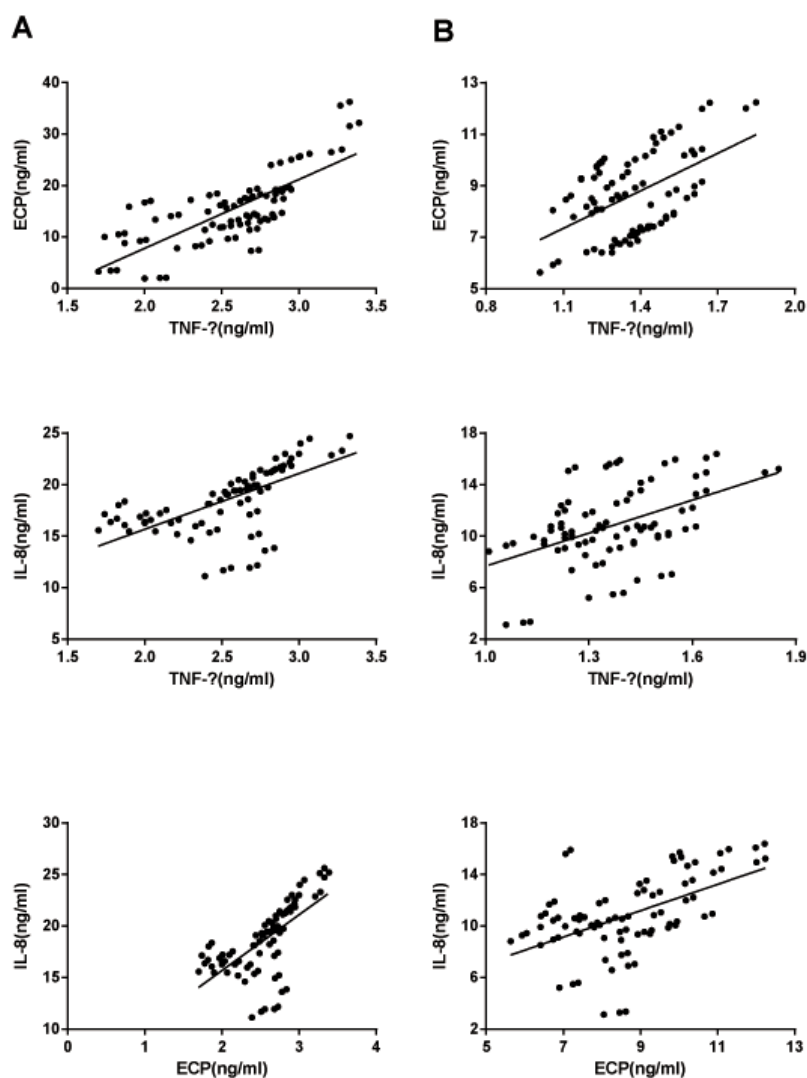


Figure 1. Correlation analysis of serum TNF- α , IL-8, and ECP levels of patients in acute attack and clinical remission (A: acute attack; B: clinical remission) Note: TNF, tumor necrosis factor; ECP, eosinophil cationic protein; IL, interleukin.

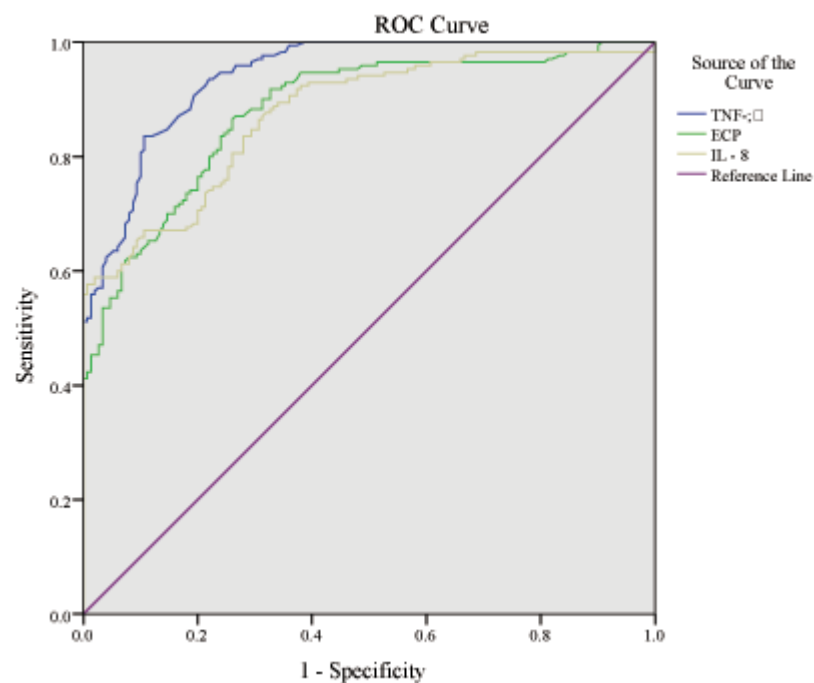


Figure 2. ROC curves of serum TNF- α , IL-8, and ECP levels in diagnosis of asthma

Note: TNF, tumor necrosis factor; ECP, eosinophil cationic protein; IL, interleukin; ROC, receiver operating characteristic.