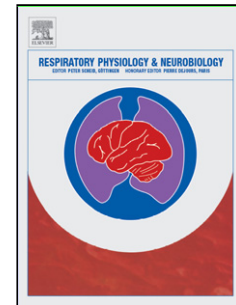


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Tumor necrosis factor- α , interleukin-8 and eosinophil cationic protein as serum markers of glucocorticoid efficacy in the treatment of bronchial asthma

Running title: TNF- α , IL-8 and ECP as serum markers in BA

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Highlights

- Correlations between TNF- α , ECP, IL-8 levels and BA are studied.
- TNF- α , ECP, IL-8 are negatively related to lung functions in BA patients.
- TNF- α , ECP, IL-8 levels are positively correlated with clinical stages of BA.
- TNF- α , ECP, IL-8 level are positively correlated with severity of BA.
- TNF- α , ECP, IL-8 are markers for evaluating efficacy of glucocorticoid in BA.

ABSTRACT

Background: Bronchial asthma (BA) is a common chronic respiratory disease that has exhibited a rising global incidence in recent years. Glucocorticoids are used for the treatment of BA. Emerging evidence has demonstrated the roles of tumor necrosis factor (TNF- α), interleukin-8 (IL-8) and eosinophil cationic protein (ECP) in BA. The present study investigated whether TNF- α , IL-8 and ECP were associated with the clinical stages and severity of BA and the efficacy of glucocorticoids in the treatment of BA.

Methods: A total of 199 patients with BA and 174 healthy individuals were included in this study. Patients with BA underwent glucocorticoid treatment, and the TNF- α , IL-8 and ECP levels and lung functions of the subjects were measured. The correlations of the TNF- α , IL-8 and ECP levels with BA severity, clinical staging and lung functions were assessed. We investigated whether the TNF- α , IL-8 and ECP levels aided in evaluating the efficacy of using glucocorticoids for the treatment of BA.

Results: TNF- α , IL-8 and ECP exhibited high levels in patients with BA, and glucocorticoid treatment notably decreased these levels. The TNF- α , IL-8 and ECP levels were positively correlated with the clinical stages and severity of BA and negatively correlated with lung function. TNF- α , IL-8 and ECP can be used as serum markers to predict the efficacy of glucocorticoids in the

treatment of BA.

Conclusion: The key findings of this study collectively support a role for TNF- α , IL-8 and ECP in BA development, and TNF- α , IL-8 and ECP can be used as serum markers of glucocorticoid efficacy in BA.

Keywords: Tumor necrosis factor- α ; Interleukin-8; Eosinophil cationic protein; Bronchial asthma; Clinical stages; Severity; Glucocorticoid; Serum marker

1. Introduction

Bronchial asthma (BA) is a chronic inflammatory disease of the respiratory system that contributes to an imbalance in the oxidant-antioxidant capacity of the lungs (Gvozdjakova et al., 2005). BA has a multifactorial etiology (Panek et al., 2012), and genetic factors and inappropriate immune responses to environmental allergens are contributors (Takagi et al., 2011; Karam et al., 2012). Patients with severe BA may experience pulmonale later in life (Shedeed, 2010) and face difficulty because of the requisite extensive diagnostic assessment, personalized treatment and high cost of health-care resources (Lommatzsch and Virchow, 2014). Many patients with severe asthma remain in need of regular treatment with oral glucocorticoids despite high-dose inhalational therapy (Bel et al., 2014). Prolonged glucocorticoid use improves the outcomes of acute respiratory distress syndrome (Meduri et al., 2016). And existing evidence showed that Glucocorticoids may be a potent treatment for BA (Matsumura, 2010).

Elevated serum levels of tumor necrosis factor- α (TNF- α), interleukin-8 (IL-8) and eosinophil cationic protein (ECP) are involved in the progression and development of BA (Jiang et al., 2018). Serum (S-ECP) levels are a biomarker of asthma because it reflects the degree of bronchial eosinophilic inflammation (Prehn et al., 1998). TNF- α expression also upregulates the expression of adhesion molecules that contribute to airway inflammation (Babu et al., 2011). IL-8 is elevated in

asthmatic airways (Ge et al., 2010), which suggests that highly expressed IL-8 participates in asthma development. TNF- α reduced the responsiveness to glucocorticoids in a mouse model of neutrophilic airway inflammation (Dejager et al., 2015). Glucocorticoids inhibit IL-8 expression in human pulmonary microvascular endothelial cells and children with primary nephrotic syndrome (Shi et al. 2014; Guan et al. 2018). Decreased ECP enhanced the beneficial efficacy of topical glucocorticoid treatment (Benson et al., 2000). We hypothesized that TNF- α , IL-8, and ECP may play a significant role in BA and BA treatment with glucocorticoids. The present study investigated the correlation of TNF- α , IL-8, and ECP with the clinical staging and severity of BA and the efficacy of glucocorticoids in the treatment of BA.

2. Materials and methods

2.1 Ethics statement

The Ethics Committee of Zhangjiagang First People's Hospital and Dongchangfu People's Hospital of Liaocheng approved this study. Informed consent was obtained from all study subjects and/or their legal guardians.

2.2 Study Subjects

A total of 199 BA patients who received treatment in the Outpatient with Respiratory Diseases and were hospitalized in Zhangjiagang First People's Hospital or Dongchangfu People's Hospital of Liaocheng between June 2015 and July 2016 were included in this study as the BA group. A total of 112 males and 87 females with a mean age of 47.4 ± 4.5 year (range 34 to 60 years) were enrolled. BA was classified into acute episode, chronic persistent or clinical remission based on different stages according to the *Chinese Guideline for the Prevention and Management of Bronchial Asthma* (2013). Sixty-five patients were in an acute episode, 46 patients were in a chronic persistent state, and 88 patients were in clinical remission. Twenty-five patients were in a severe stage of acute episode of BA, 22 patients were in a moderate stage, and 18 patients were in a mild stage (Liu,

2015). Patients in line with the following criteria were included in this study (Misra et al., 2015): (1) patients who suffered from wheezing, chest tightness, shortness of breath, and cough induced by anaphylactogen, cold air, or physical or chemical stimulation; (2) patients who had sporadic or diffuse wheezing with exhalation that could be heard in lungs; (3) patients who had the aforementioned symptoms that could be relieved voluntarily or with treatment; and (4) patients who had wheezing, chest tightness, shortness of breath, or cough caused by other diseases. Patients in line with the following criteria were excluded: (1) patients treated with systemic hormones, immunoglobulins or immunosuppressant in the past 3 months; (2) patients with other autoimmune diseases; (3) patients with chronic obstructive pulmonary disease, pulmonary tuberculosis, lung cancer or other lung diseases; (4) patients complicated with infectious disease and treated with antibiotics but not receiving adrenocorticosteroids or other immunomodulators (Ordonez et al., 2000); and (5) patients in the BA and control groups who had chronic obstructive pulmonary disease, pulmonary infection, interstitial pneumonia, allergic constitution, hepatitis, various immune system diseases, advanced tumor, acute or chronic leukemia, pregnant or lactating women, or received organ transplantation. A total of 174 healthy individuals (88 males and 86 females aged 33 to 62 years, with a mean age of 46.6 ± 5.3 years) were enrolled as control subjects. None of the controls were blood related to the BA group, and there were no significant differences in age or sex.

2.3 Specimen treatment

Fasting venous blood (5 mL) was taken from vein at the elbow in the morning. Serum was placed into a tube with natrium citricum anticoagulant, sufficiently mixed, and allowed to stand for 30 min. Serum was centrifuged in a high-speed centrifuge (Avanti J-26X, Beckman Coulter Life Sciences, Brea, CA, USA) at 36 g for 10 min. The supernatant serum was obtained and placed in an ultra-low temperature refrigerator (DW-86L388A, Haier, China) for further use.

2.4 Enzyme-linked immunosorbent assay (ELISA)

ELISA was used to determine the contents of TNF- α , ECP, and IL-8. Kits for TNF- α and IL-8 were purchased from Yes Biotech Laboratories (Mississauga, Canada). An ECP ELISA kit and IL-8, TNF- α and ECP standard products were purchased from Pharmasia Company (Uppsala, Sweden). All patients received long-term inhaled glucocorticoid treatment (BATCHVF602, budesonide dry powder inhalant, AstraZeneca AB, Lund, Sweden, 200 to 400 g, twice daily) for 8 weeks. Other asthma control drugs were avoided during the treatment period. Changes in serum TNF- α , IL-8 and ECP levels were measured once weekly. Levels were determined within one day after the serum was separated. Experiments were performed in strict accordance with product instructions.

2.5 Detection of lung function

The ratio between forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) was measured using a PFT multifunctional lung function instrument (JEGER, Berlin, Germany). The best value of 3 repeated measurements was taken (the value change was less than 5% each time). The ratio between FEV₁ and estimated value (FEV₁%) and the ratio between FEV₁ and FVC were recorded. An impulse oscillometry system (IOS, JEGER, Germany) was used to detect respiratory impedance (Z5), airway resistance in 5 Hz (R5), airway resistance in 20 Hz (R20), electrical impedance in 5 Hz (X5), resonant frequency (Fres), central airway resistance (RC), and peripheral airway resistance (RP). The instrument error range was less than 3%, and the accuracy was 0.05 L. Detections were repeated 3 times.

2.6 Diagnosis of therapeutic efficacy

The following requirements were considered as effective glucocorticoid treatment for BA: (1) no emergency caused by asthma; (2) rare attacks of asthma; (3) no chronic symptoms, including nocturnal symptoms; (4) need for use of glucocorticoid for treatment; (5) no restrictions on activities, including sports; and (6) no or few drug adverse reactions. If one of these events occurred, and then the response was considered invalid. These criteria identified 167 cases as effective and 32

cases as invalid after treatment.

2.7 Statistical analysis

SPSS 21.0 (IBM Corp. Armonk, NY, USA) was used for statistical analysis. Enumeration data are expressed as ratios or percentages, and pairwise comparisons between groups were analyzed using chi-square tests. Measurement data are expressed as the means $\pm$ standard deviation. Comparisons between two groups were analyzed using the independent sample *t*-test, and measurement data were analyzed using one-way analysis of variance (ANOVA). Spearman correlation coefficient was used for correlation analysis of TNF- α , ECP and IL-8 levels with BA clinical staging and severity. Pearson's correlation analysis was used to determine the changing relationships between indexes of renal function. Receiver operating characteristic (ROC) curves were used to evaluate correlations between TNF- α , ECP, IL-8 and the efficacy of glucocorticoids for the treatment of BA. All statistical tests were performed using two-sided probability tests, and *p*-values < 0.05 indicated statistically significant differences.

3. Results

3.1 TNF- α , IL-8 and ECP levels are higher in BA patients but decreased after glucocorticoid treatment

ELISA was used to determine TNF- α , IL-8 and ECP levels. TNF- α , IL-8 and ECP levels of BA patients in acute episode, chronic persistent and clinical remission were higher than the control group prior to inhaled glucocorticoid treatment ($p < 0.05$). TNF- α , IL-8 and ECP decreased after treatment in patients in acute episode, chronic persistent and clinical remission ($p < 0.05$) (Table 1). Taken together, glucocorticoids attenuated TNF- α , IL-8 and ECP levels in BA patients.

3.2 TNF- α , IL-8 and ECP levels are decreased in BA patients in acute episode after glucocorticoid treatment

TNF- α , IL-8 and ECP levels in patients in severe, moderate, and mild stages of acute episode were higher than the control group prior to treatment ($p < 0.05$). TNF- α , IL-8 and ECP levels in patients in severe, moderate, and mild stages of acute episode exhibited a decreasing trend after treatment ($p < 0.05$). TNF- α , IL-8 and ECP levels in patients in the 3 different stages in acute episode decreased after a 2-week glucocorticoid treatment ($p < 0.05$). Glucocorticoids attenuated TNF- α , IL-8 and ECP levels in BA patients in different stages of acute episode (Table 2).

3.3 TNF- α , IL-8 and ECP levels positively correlate with BA clinical stages and severity

Correlation analysis was used to investigate the relationship between TNF- α , IL-8 and ECP levels and BA clinical stages and severity. The results revealed that TNF- α , IL-8 and ECP levels positively correlated with BA clinical stages ($r = 0.482$, $p < 0.001$; $r = 0.558$, $p < 0.001$; $r = 0.464$, $p < 0.001$) and severity ($r = 0.508$, $p < 0.001$; $r = 0.783$, $p < 0.001$; $r = 0.494$, $p < 0.001$). TNF- α , IL-8 and ECP levels positively correlated with BA clinical stages and severity (Table 3).

3.4 TNF- α , IL-8 and ECP levels negatively correlate with lung functions of BA patients

Lung function detection using an impulse oscillometry system (IOS) was performed for correlational analysis between TNF- α , IL-8 and ECP levels and lung function in BA patients. The results revealed that FEV₁%pred (%), FEV₁/FVC and X5 (kPa.L⁻¹.S⁻¹) were lower in patients compared to the control group prior to treatment (Table 4). Z5 (kPa.L⁻¹.S⁻¹), R5%pred (%), R20%pred (%), Fres (HZ), Rc (kPa.L⁻¹.S⁻¹) and Rp (kPa.L⁻¹.S⁻¹) were greater in patients compared to the control group prior to treatment ($p < 0.05$). Pearson's correlation analysis was used to investigate correlation analysis between serum TNF- α , IL-8 and ECP levels and lung function indexes. Lung function indexes FEV₁%pred (%) and FEV₁/FVC and IOS index X5 (kPa.L⁻¹.S⁻¹) negatively correlated with TNF- α , IL-8 and ECP levels. IOS indexes Z5%pred (%), R5%pred (%), R20%pred (%), Fres (HZ), Rc (kPa.L⁻¹.S⁻¹) and Rp (kPa.L⁻¹.S⁻¹) positively correlated with TNF- α , IL-8 and ECP levels. TNF- α , IL-8 and ECP levels negatively correlated with the lung functions of

BA patients (Table 5).

3.5 TNF- α , IL-8 and ECP are serum markers of glucocorticoid efficacy for BA treatment

ROC curve (Fig. 1) evaluated whether TNF- α , IL-8 and ECP reflected the efficacy of glucocorticoids for the treatment of BA. The diagnostic threshold of IL-8 was 5.065 (ng/mL), the area under curve was 0.752, specificity was 86.8%, and sensitivity was 56.3%. The diagnostic threshold of TNF- α was 4.075 (ng/mL), the area under curve was 0.912, specificity was 85.6%, and sensitivity was 96.9%. The diagnostic threshold of ECP was 8.415 (ng/mL), the area under curve was 0.772, specificity was 57.5% and sensitivity was 87.5%. These results demonstrated that TNF- α , IL-8 and ECP may be used as serum markers to predict glucocorticoid efficacy for BA treatment.

4. Discussion

BA is a chronic inflammatory condition that is accompanied by increased cardiovascular events (Wang et al., 2013). The current study demonstrated that serum levels of TNF- α , IL-8, and ECP positively correlated with the clinical stages and severity of BA, and these proteins may be used as biomarkers to evaluate glucocorticoid efficacy in the treatment of BA.

Our results indicated that TNF- α , IL-8, and ECP levels were higher in BA patients compared to healthy subjects, and these levels positively correlated with the clinical stages and severity of BA. IL-8 is an effective neutrophil attractant and activator that affects acute lung injury via the formation of autoantibodies (Allen and Kurdowska, 2014). IL-8 is upregulated in severe asthma, and it plays a significant role in the accumulation of neutrophils (Nakagome and Nagata, 2011), which aid in the activation of immunocompetent cells in asthma (Ciepiela et al., 2015). IL-8 may allow eosinophils to accumulate in the airways of asthma patients and promote disease progression (Kawashima et al., 2013). TNF- α plays a significant role in building a complex inflammation-cancer link (Wu and Zhou, 2010). Genetic variations in TNF- α may be responsible for the occurrence of childhood wheezing and asthma (Shaker et al., 2013). TNF- α is associated

with the pathogenesis of asthma (Dejager et al., 2015). TNF- α is an important agent in airway inflammation (Lee et al., 2016), and it plays a significant role in promoting BA development (Nakagome and Nagata, 2011). TNF- α inhibited the apoptosis of human eosinophils (Kankaanranta et al., 2014) and mediated the resolution of inflammation (Tani et al., 2014). TNF- α also regulated ECP-induced apoptosis (Chang et al., 2010). ECP is a cytotoxic, pro-fibrotic ribonuclease that plays a role in eosinophil-related diseases, and it may be used to monitor these diseases in combination with blood eosinophilias (Bystrom et al., 2012). Our study revealed that ECP levels increased in patients with BA, which indicates activation of eosinophils in patients with BA. Therefore, our study concluded that TNF- α , IL-8, and ECP participated in BA development.

TNF- α , IL-8, and ECP function as serum markers to predict the efficacy of glucocorticoids in the treatment of BA. TNF- α mediates glucocorticoid synthesis in the adrenal glands of mice acutely infected with *Trypanosoma cruzi* (Villar et al., 2013). Previous studies found that dexamethasone (a glucocorticoid) down-regulated IL-8 (Shi et al., 2014). One study suggested that topical glucocorticoid treatment decreased the proportion of eosinophils, ECP levels, and the secretory response to methacholine compared to placebo (Klementsson et al., 1991). These studies indicated that glucocorticoid was associated with TNF- α , IL-8, and ECP. Recent findings also demonstrated that increased serum levels of TNF- α , IL-8, and ECP were implicated in the development and progression of BA (Jiang et al., 2018). TNF- α , IL-8, and ECP levels positively correlated to the clinical stages and severity of BA, and the aforementioned findings supported our conclusion that TNF- α , IL-8, and ECP may be used as serum markers for predicting glucocorticoid efficacy in the treatment of BA.

5. Conclusion

In conclusion, our study demonstrated that the TNF- α , IL-8, and ECP levels were positively correlated with the clinical stages and severity of BA, providing evidence that TNF- α , IL-8, and ECP could serve as novel markers for the treatment and evaluation of BA. However, further studies

are required to validate our findings because of the limited sample size and experimental conditions of our study.

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Disclosure statement

None

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Figure Legends

Fig. 1. TNF- α , IL-8 and ECP function as serum markers for predicting the glucocorticoid efficacy in the treatment of BA. ROC, receiver operating characteristic; TNF- α , tumor necrosis factor- α ; ECP, eosinophil cationic protein; IL-8, interleukin-8.

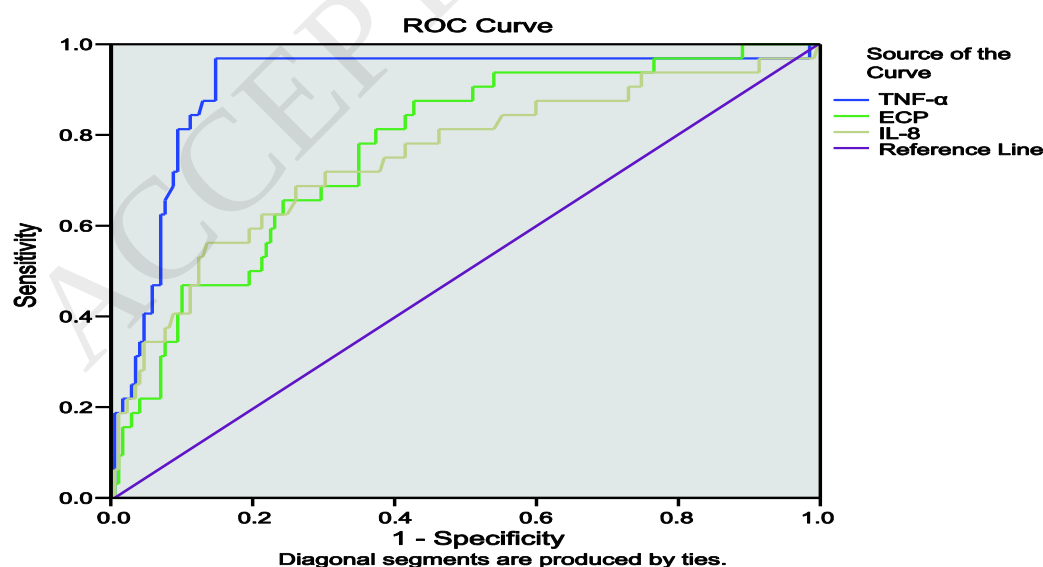


Table 1

TNF- α , IL-8 and ECP levels are higher in patients with BA in acute episode, chronic persistent and clinical remission but decreased after treatment with glucocorticoid.

Groups	control	acute episode	chronic persistent	clinical remission
TNF- α (ng/ml)				
Before treatment	2.31 $\pm$ 1.05	7.86 $\pm$ 4.66*	5.24 $\pm$ 2.72*#	3.51 $\pm$ 1.92*#&
After treatment	—	5.67 $\pm$ 3.29 [^]	3.28 $\pm$ 1.54 [^]	1.34 $\pm$ 0.43 [^] #&
ECP (μ g/L)				
Before treatment	3.78 $\pm$ 1.32	23.66 $\pm$ 10.29*	18.12 $\pm$ 6.33*#	12.04 $\pm$ 5.21*#&
After treatment	—	17.36 $\pm$ 7.09 [^]	9.29 $\pm$ 5.60 [^]	5.37 $\pm$ 3.14 [^] #&
IL-8 (ng/L)				
Before treatment	2.34 $\pm$ 0.84	8.12 $\pm$ 5.69*	5.25 $\pm$ 2.88*#	3.13 $\pm$ 1.11*#&
After treatment	—	5.45 $\pm$ 4.09 [^]	3.39 $\pm$ 1.94 [^]	1.59 $\pm$ 0.82 [^] #&

Note: *, $p < 0.05$ vs. the control group; #, $p < 0.05$ vs. the patients in acute episode; &, $p < 0.05$ vs. the patients in chronic persistent; [^], $p < 0.05$ vs. before treatment; TNF- α , tumor necrosis factor-alpha; ECP, eosinophil cationic protein; IL-8, interleukin-8. The data were presented as mean $\pm$ standard deviation, and analyzed by one-way ANOVA.

Table 2

TNF- α , IL-8 and ECP levels are decreased in patients with BA in acute episode with varying severity after treatment with glucocorticoid.

Groups	control group	mild stage	moderate stage	severe stage
TNF- α (ng/ml)				
Before treatment	2.31 $\pm$ 1.05	4.94 $\pm$ 2.24 [*]	6.85 $\pm$ 3.17 ^{*#}	10.85 $\pm$ 5.37 ^{*#&}
After treatment	—	3.44 $\pm$ 2.00 [^]	4.67 $\pm$ 2.34 ^{#^}	8.16 $\pm$ 3.18 ^{#&^}
ECP (μ g/L)				
Before treatment	3.78 $\pm$ 1.32	14.73 $\pm$ 4.80 [*]	19.52 $\pm$ 5.53 ^{*#}	33.74 $\pm$ 7.62 ^{*#&}
After treatment	—	10.18 $\pm$ 3.32 [^]	16.25 $\pm$ 4.84 ^{#^}	23.51 $\pm$ 5.17 ^{#&^}
IL-8 (μ g/L)				
Before treatment	2.34 $\pm$ 0.84	4.94 $\pm$ 3.08 [*]	6.40 $\pm$ 4.32 ^{*#}	11.92 $\pm$ 6.17 ^{*#&}
After treatment	—	2.38 $\pm$ 1.43 [^]	4.29 $\pm$ 2.17 ^{#^}	8.69 $\pm$ 4.45 ^{#&^}

Note: *, $p < 0.05$ vs. the control group; #, $p < 0.05$ vs. the patients in mild stage; &, $p < 0.05$ vs. the patients in moderate stage; ^, $p < 0.05$ vs. before treatment. TNF- α , tumor necrosis factor- α ; ECP, eosinophil cationic protein; IL-8, interleukin-8. The data were presented as mean $\pm$ standard deviation and analyzed by one-way ANOVA.

Table 3

The TNF- α , IL-8 and ECP levels are positively correlated with clinical stages and severity of BA.

	TNF- α		ECP		IL-8	
	r	<i>p</i>	r	<i>p</i>	r	<i>p</i>
Clinical stages	0.482	< 0.001	0.558	< 0.001	0.464	< 0.001
Severity	0.508	< 0.001	0.783	< 0.001	0.494	< 0.001

Note: TNF- α , tumor necrosis factor- α ; ECP, eosinophil cationic protein; IL-8, interleukin-8. The data were presented as mean $\pm$ standard deviation and analyzed by *t*-test.

Table 4

FEV₁%pred (%), FEV₁/FVC and X5 (kPa.L⁻¹.S⁻¹) are decreased in patients before treatment when compared with those in patients in the control group

Groups	FEV ₁ %pred (%)	FEV ₁ /FVC	Z5 (kPa.L ⁻¹ .S ⁻¹)	R5%pred (%)	R20%pred (%)	X5 (kPa.L ⁻¹ .S ⁻¹)	Fres (HZ)	Rc (kPa.L ⁻¹ .S ⁻¹)	Rp (kPa.L ⁻¹ .S ⁻¹)
Control group	94.12 ± 7.32	86.54 ± 5.78	0.37 ± 0.15	128.52 ± 25.14	109.54 ± 10.11	-0.32 ± 0.13	12.32 ± 2.33	0.15 ± 0.03	0.23 ± 0.06
BA group	84.22 ± 10.49*	77.98 ± 9.31*	0.63 ± 0.33*	152.67 ± 36.64*	123.54 ± 18.22*	-0.49 ± 0.25*	17.16 ± 6.74*	0.38 ± 0.20*	0.44 ± 0.22*

Note: FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; Z5, respiratory impedance; R5, airway resistance in 5Hz; R20, airway resistance in 20Hz; X5, electrical impedance in 5Hz, Fres, resonant frequency, RC, central airway resistance, RP, peripheral airway resistance. *, $p < 0.05$ vs. the control group. The measurement was repeated at least 3 times. The data were presented as mean ± standard deviation, and analyzed by *t*-test

Table 5

Lung function detection shows that the TNF- α , IL-8 and ECP levels are negatively correlated with lung functions of patients with BA.

Indices	FEV1% pred (%)	FEV ₁ /F VC	Z5 (kPa.L ⁻¹ .S ⁻¹)	R5%p red (%)	R20%p red (%)	X5 (kPa.L ⁻¹ .S ⁻¹)	Fres (HZ)	Rc (kPa.L ⁻¹ .S ⁻¹)	Rp (kPa.L ⁻¹ .S ⁻¹)	
TNF- α	r	-0.53	-0.537	0.385	0.278	0.282	-0.278	0.258	0.436	0.412
	p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001
ECP	r	-0.564	-0.526	0.453	0.317	0.331	-0.385	0.394	0.475	0.404
	p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001
IL-8	r	-0.506	-0.439	0.333	0.213	0.338	-0.264	0.29	0.40	0.282
	p	< 0.001	< 0.001	< 0.001	0.003	< 0.001	< 0.001	0.001	< 0.001	< 0.001

Note: FEV1, forced expiratory volume in one second; FVC, forced vital capacity; Z5, respiratory impedance; R5, airway resistance in 5Hz; R20, airway resistance in 20Hz; X5, electrical impedance in 5Hz, Fres, resonant frequency, RC, central airway resistance, RP, peripheral airway resistance. The measurement was repeated at least 3 times. The data were presented as mean $\pm$ standard deviation, and analyzed by one-way ANOVA.