Inflammation and Cell Signaling 2022; 9: e1210. doi: 10.14800/ics.1210; © 2022 by Yaqin Liu, *et al*.
http://www.smartscitech.com/index.php/ics

RESEARCH ARTICLE

A study on the significance of anti-endothelial cell antibodies in chronic obstructive pulmonary disease and the effect of methylprednisolone intervention

Yaqin Liu[1], Li Ma[2,*]

[1] *Department of Respiratory and Critical Care Medicine, Guizhou Provincial People's Hospital, Guiyang, Guizhou 550002, China*
[2] *Department of Respiratory and Critical Care Medicine, Guizhou Provincial People's Hospital, Guiyang, Guizhou 550002, China*

[*] Correspondence: Li Ma
E-mail: maligy@126.com
Received: Jun 28, 2022
Published: Aug 24, 2022

The purpose was to confirm the significance of Anti-endothelial cell antibodies (AECA) in chronic obstructive pulmonary disease (COPD) and validate the effect of methylprednisolone intervention. We recruited 40 patients with stable COPD, 40 patients with an acute exacerbation of COPD, and 20 healthy volunteers from March 2019 to August 2021. The healthy volunteers constituted the healthy control group. All patients with stable COPD were divided into the mild-moderate COPD group and the severe-very severe COPD group, for whom AECA and vascular endothelial growth factor (VEGF) in peripheral blood were detected by ELISA. The patients with acute exacerbation of COPD were divided into routine treatment group and methylprednisolone group by random number method. The routine group received routine treatment, and the methylprednisolone group was treated with methylprednisolone on the basis of routine treatment, and the course of treatment was 1 week, respectively. The AECA and VEGF in the peripheral blood of the two groups of patients before and after treatment were detected by ELISA. Compared to control group, the AECA concentration was significantly elevated as the condition of COPD got serious between mild-moderate COPD group and the severe-very severe COPD group($P<0.05$). And VEGF concentration was significantly lower as the condition of COPD got serious ($P<0.05$). AECA concentration was significantly lower after methylprednisolone treatment than before in patients with COPD exacerbation, and significantly lower than patients receiving the routine treatment ($P<0.05$). Besides, VEGF concentration was significantly elevated in patients with COPD exacerbation after methylprednisolone treatment than before, and considerably higher than patients receiving the routine treatment ($P<0.05$). AECAs may be involved in the occurrence and development of COPD and related to its severity. Methylprednisolone can help reduce AECA expression while promoting VEGF expression.

Keywords: anti-endothelial cell antibodies, chronic obstructive pulmonary disease, methylprednisolone, vascular endothelial growth factor, glucocorticoids

Abbreviations: COPD, Chronic obstructive pulmonary disease; AECA, Anti-endothelial cell antibodies; VEGF, Vascular endothelial growth factor; ELISA, Enzyme linked immunosorbent assay; ANOVA, Analysis of Variance; GCs, Glucocorticoids; FEV1, Forced expiratory volume in one second; FVC, Forced Vital Capacity

To cite this article: Yaqin Liu, *et al.* A study on the significance of anti-endothelial cell antibodies in chronic obstructive pulmonary disease and the effect of methylprednisolone intervention. Inflamm Cell Signal 2022; 9: e1210. doi: 10.14800/ics.1210.

Introduction

Chronic obstructive pulmonary disease (COPD) is usually presented with airflow obstruction and continued respiratory symptoms[1]. COPD is mainly related to the abnormal inflammatory response of the lungs to smoking or other noxious gases or particles[2]. COPD is known for its high prevalence and mortality, which causes an enormous social and economic burden on society at large[1,2]. The pathogenesis of COPD is not fully clarified yet. Chronic airway and pulmonary inflammation, protease-antiprotease imbalance, and oxidation/antioxidation imbalance have been identified as the primary pathogenic factors[3-5]. Apart from these, autoimmune response, alveolar septal cell apoptosis, and genetic polymorphism have attracted growing attention as new pathogenic factors of COPD.

Anti-endothelial cell antibodies (AECAs) can recognize antigen molecules on the surface of or inside endothelial cells, affecting the endothelial function[6]. AECAs can bind to endothelial cells to induce an immune response, leading to vascular injury[6,7]. AECAs are markers of vasculitis and vascular injury, and are widely presented in various autoimmune diseases associated with immune- and inflammation-mediated vascular wall injury[8]. The above facts indicate that the production of AECAs may be one crucial factor inducing the autoimmune response. Many studies have suggested that the occurrence and development of COPD are closely related to the autoimmune response[9-10], and the expression of AECAs has been found related with emphysema closely[11,12]. As reported, as the AECA concentration increased, the alveolar septal cell apoptosis increased significantly, which aggravates emphysema[12]. From the above, we infer that AECAs may be involved in emphysema. To discuss the relationship between AECAs and emphysema, Yao, *et al*[13] built a rat model of smoking-induced emphysema, finding that AECA induced alveolar septal cell apoptosis by inhibiting vascular endothelial growth factor (VEGF) and VEGFR-2 expression. Their study[13] further found that the AECA expression decreased significantly in the serum of rats after methylprednisolone treatment. The above result indicated that methylprednisolone inhibited AECA expression, blocking the AECA-induced autoimmune mechanism and alveolar septal cell apoptosis, thereby preventing emphysema[13].

In the present study, we determined the AECA and VEGF concentrations in the peripheral blood of COPD patients and compared the results before and after methylprednisolone treatment. The purpose was to confirm the significance of AECA in COPD and validate the effect of methylprednisolone intervention. Our findings will lay the foundation for further exploration of COPD's pathogenesis and treatment pathways.

Patients and Methods

The present study was approved by the ethics committee of our hospital. We recruited 40 patients with stable COPD, 40 patients with an acute exacerbation of COPD, and 20 healthy volunteers from March 2019 to August 2021. The healthy volunteers constituted the healthy control group. All patients with stable COPD received a pulmonary function test. They were divided into the mild-moderate COPD group and the severe-very severe COPD group based on the FEV1/FVC ratio and FEV_1/pred% obtained by the pulmonary function test. The severity grading of COPD was performed using the following criteria (pulmonary function test after inhalation of the bronchodilators): mild: $FEV_1/FVC<70\%$,$FEV_1/pred\%\geq80\%$; moderate: $FEV_1/FVC<70\%$, $50\%\leq FEV_1/pred\%<80\%$; severe: $FEV_1/FVC<70\%$, $30\%\leq FEV_1/pred\%<50\%$; very severe: $FEV_1/FVC<70\%$, $FEV_1/pred\%<30\%$ or $FEV_1/pred\%<50\%$ combined with respiratory failure.

Inclusion criteria for COPD patients: (1) Conforming to the diagnostic criteria developed by the Chinese Medical Association in the Guidelines for the Diagnosis and Management of COPD (Revised Version 2021)[2]; (2) Definition of COPD exacerbation: Patients with stable COPD suffered from an exacerbation of cough, expectoration, shortness of breath and/or gasping, increased sputum volume and/or changes in sputum properties. The patients might have manifestations such as fever, which indicated aggravated inflammation; (3) Stable COPD: The symptoms such as cough, expectoration, and shortness of breath were relieved considerably after treatment. The patient generally recovered to the pre-exacerbation condition.

Exclusion criteria: (1) Having received systemic use of glucocorticoids within 1 week before the hospital visit; (2) combined with systemic fungal infection; (3) combined with significant gastrointestinal bleeding; (4) combined with other chronic diseases, such as hypertension, diabetes and coronary heart disease; (5) combined with bronchodilation, bronchial asthma, and pulmonary hypertension; (6) combined with some symptomatic infectious diseases, such as tuberculosis.; or some symptomatic viral diseases, such as herpes affecting the eyes and herpes zoster; (7) combined with diseases that affected the study results, such as rheumatic immune diseases, malignancies and metabolic diseases.

A random number method was used to divide COPD patients into the methylprednisolone and the routine treatment groups. The routine treatment group received standard systemic treatment according to the regimen described in the Guidelines for the Diagnosis and Management of COPD (Revised Version 2021)[2], which consisted of the following: (1) controlled oxygen therapy; (2) antibiotics; (3) bronchodilators; (4) mechanical ventilation. The methyl-prednisolone treatment group received an extra intravenous drip of methylprednisolone 40 mg once daily based on the standard systemic treatment. The treatment lasted for one week in either group.

Table 1. The characteristics in the healthy control group, mild-moderate COPD group, and severe-very severe COPD group.

Index	control group	mild-moderate COPD group	severe-very severe COPD group	P-value
Gender				
Male	10	11	13	>0.05*,#
Female	10	7	9	>0.05*,#
Age	63.51±9.30	62.43±7.73	70.22±8.35	>0.05*,#
Smoking index (number/year)	547.21±176.58	676.24±132.24	653.65±128.48	>0.05*,#
Disease duration (years)	N/A	7.2±1.3	6.8±2.4	>0.05#
AECA concentration（$ng.ml^{-1}$）	257.51±96.30	576.31±73.07	643.79±69.13	0.00&, 0.012&
VEGF concentration（$pg.ml^{-1}$）	1397.56±186.75	850.18±163.73	715.45±161.82	0.00&, 0.017&

*P>0.05 in mild-moderate COPD group compared with healthy control group, #P>0.05 in severe-very severe COPD group compared with mild-moderate COPD group, &P<0.05 by pairwise comparison.

Table 2. The characteristics in the methylprednisolone intervention group and the control group.

Index	routine treatment group	methylprednisolone group	P-value
Gender			
Male	10	12	>0.05*
Female	10	8	>0.05*
Age	69.54±12.67	71.76±9.23	>0.05*
Smoking index (number/year)	679.28±133.47	634.66±142.75	>0.05*
Disease duration (years)	6.3±1.4	5.6±1.5	>0.05*

Compared with routine treatment group, *P>0.05.

Table 3. AECA concentration ($ng.ml^{-1}$) changes before and after treatment.

Index	AECA concentration($ng.ml^{-1}$)	P-value
Routine treatment group		
before	613.55±76.04	>0.05*
after	606.56±77.80	0.085**
methylprednisolone group		
before	612.42±55.52	0.00***
after	486.38±90.33	0.00****

Methylprednisolone group before treatment compared with routine treatment group before treatment, *P>0.05; conventional group before and after treatment, **P>0.05; methylprednisolone group before and after treatment comparison, ***P<0.01; methylprednisolone group after treatment by comparison to routine treatment group after treatment, ****P<0.01.

Table 4. VEGF concentration ($pg.ml^{-1}$) changes before and after treatment.

Index	VEGF concentration($ng.ml^{-1}$)	P-value
Routine treatment group		
before	786.86±172.95	>0.05*
after	798.19±174.14	0.083**
methylprednisolone group		
before	788.83±186.49	0.00***
after	1096.01±183.14	0.00****

Methylprednisolone group before treatment compared with routine treatment group before treatment, *P>0.05; conventional group before and after treatment, **P>0.05; methylprednisolone group before and after treatment comparison, ***P<0.01; methylprednisolone group after treatment by comparison to routine treatment group after treatment, ****P<0.01.

From each patient in the healthy control group, mild to moderate COPD group, severe to very severe COPD group, routine treatment group, and methylprednisolone intervention group, 5 ml of peripheral venous blood was drawn before and after treatment. The venous blood sample was centrifuged at 3000 r/s for 20 min to separate the serum. The supernatant (serum) was collected into an EP tube and preserved at -80℃ before ELISA. AECA and VEGF concentrations were determined in the serum samples.

Statistical Analysis

Statistical analyses were conducted for all data using the SPSS21.0 software, and error bars were drawn. The results were expressed as mean±standard deviation. One-way ANOVA was performed for intergroup comparisons. Pairwise comparisons were conducted between the groups using the t-test. The correlations between FEV_1/pred% and AECA and VEGF concentrations were assessed in COPD patients using Pearson's correlation analysis. The relationship between AECA and VEGF concentrations was discussed using the linear correlation analysis. P<0.05 was taken to indicate a significant difference.

Results

Characteristics of patients and controls

The basic information of patients in the healthy control group, mild-moderate COPD group, and severe-very severe COPD group is shown in Table 1. The results among the three groups did not differ significantly in age, gender, or smoking intensity (*P*>0.05). The average course of the disease was 7.2±1.3 years and 6.8±2.4 years in the mild- moderate COPD group and severe-very severe COPD group respectively, indicating no significant difference (*P*>0.05). AECA and VEGF concentrations differed in patients from the healthy control group, mild-moderate COPD group, and severe-very severe COPD group. The results in each group obeyed a normal distribution, and homogeneity of variance was observed. The one-way ANOVA revealed a significant difference in AECA and VEGF concentration across the groups (F=158.08, P=0.000 for AECA concentration; F=110.54, P=0.000 for VEGF concentration). A pairwise comparison was conducted on the AECA concentration among the groups. The AECA concentration was significantly higher in the mild-moderate COPD group and the severe-very severe COPD group compared with the healthy control group (*P*<0.01). There was a significant elevation of the AECA concentration in the severe-very severe COPD group compared with the mild-moderate COPD group (*P*<0.05) (See Table 1). A pairwise comparison was conducted on the VEGF concentration among the groups. The VEGF concentration was significantly lower in the mild-moderate COPD group and the severe-very severe COPD group compared with the healthy control group (*P*<0.01). There was a significant decrease in the VEGF concentration in the severe-very severe COPD group compared with the mild- moderate COPD group (*P*<0.05) (Table 1).

Basic information of patients in the methylprednisolone intervention group and the control group

The basic information of subjects in the methylprednisolone intervention group and the control group is shown in Table 2. The two groups did not differ significantly in age, gender, smoking intensity, or course of disease (*P*>0.05).

Effect of methylprednisolone on AECA and VEGF concentrations in patients with COPD exacerbation

The AECA concentration differed in the routine treatment group(n=20) and the methylprednisolone intervention group(n=20) before and after treatment. The one-way ANOVA indicated a significant difference (F=15.288, P=0.000). The comparison showed a significantly decreased AECA concentration after treatment than before in the methylprednisolone intervention group (P<0.01). The AECA concentration in the methylprednisolone intervention group was significantly lower after treatment than in the routine treatment group (P<0.01). However, there was no significant difference in AECA concentration in the routine treatment group before and after treatment (P>0.05) (Table 3).

The VEGF concentration differed between the routine treatment group and the methylprednisolone intervention group before and after treatment. The one-way ANOVA indicated a significant difference (F=19.570, P=0.000). Pairwise comparison across the groups showed a significant elevation of the VEGF concentration after treatment than before in the methylprednisolone intervention group (P<0.01). The VEGF concentration in the methylprednisolone intervention group was significantly higher after treatment than in the routine treatment group (P<0.01). However, there was no significant difference in the VEGF concentration in the routine treatment group before and after treatment (P>0.05) (Table 4).

Relationship between AECA and VEGF concentrations in COPD patients

A correlation analysis was conducted between AECA and VEGF concentrations in COPD patients. Both obeyed a bivariate normal distribution, and the scatter plot indicated a linear trend. Pearson's correlation analysis was conducted (r=-0.325, P=0.00). It was found that the AECA and VEGF concentrations in COPD patients were negatively correlated. That is, the VEGF concentration decreased as the AECA concentration increased in COPD patients (Fig. 1).

Relationship between FEV_1/pred% and AECA concentration of COPD patients

A correlation analysis was conducted between FEV_1/pred% and AECA concentrations of COPD patients. Both obeyed a bivariate normal distribution, and the scatter plot indicated a linear trend. Pearson's correlation analysis was conducted (r=-0.467, P=0.00). It was found that FEV_1/pred% was negatively correlated with AECA concentrations of COPD patients. That is, the severity of COPD was positively correlated with AECA concentration in COPD patients (Fig. 2).

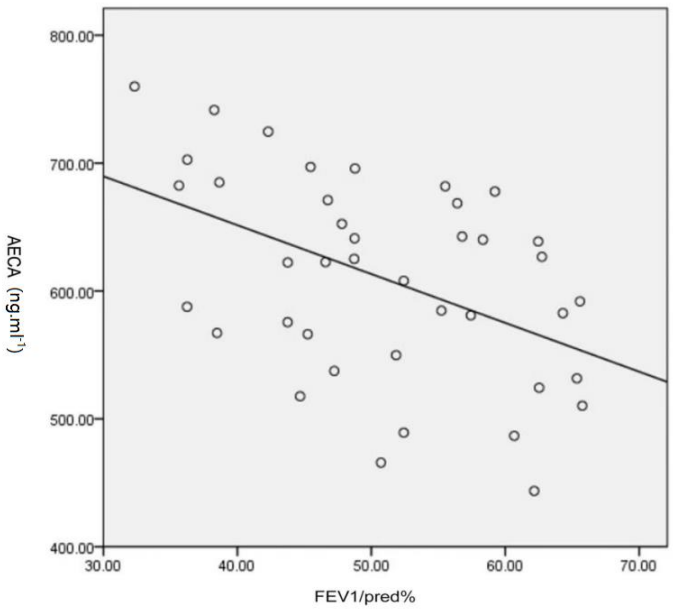

Figure 1. Scatter plot of AECA concentration and VEGF concentration in peripheral blood of patients with COPD.

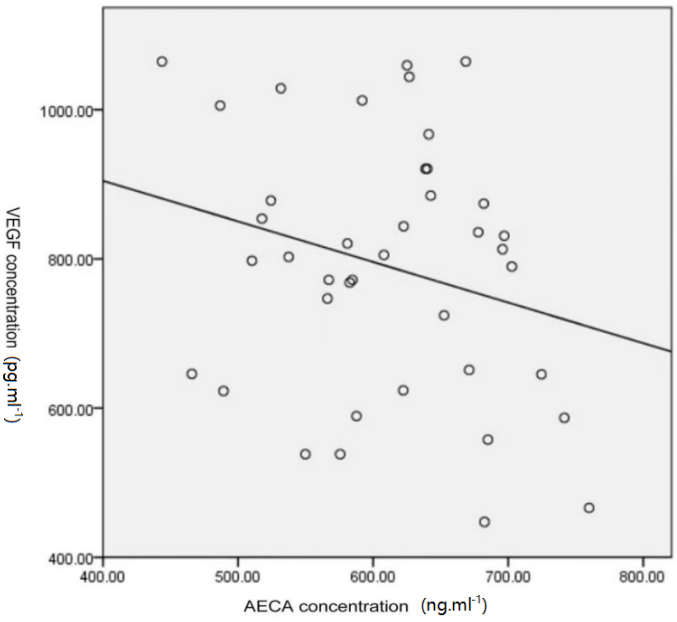

Figure 2. Scatter plot of FEV1/pred% and AECA concentration in peripheral blood in patients with COPD.

Relationship between FEV_1/pred% and VEGF concentration of COPD patients

A correlation analysis was conducted between FEV_1/pred% and VEGF concentration of COPD patients. Both obeyed a bivariate normal distribution, and the scatter plot indicated a linear trend. Pearson's correlation analysis (r=0.678, P=0.00) indicated that FEV_1/pred% was positively correlated with VEGF concentrations of COPD patients. The severity of COPD was negatively correlated with VEGF concentration in COPD patients (Fig. 3).

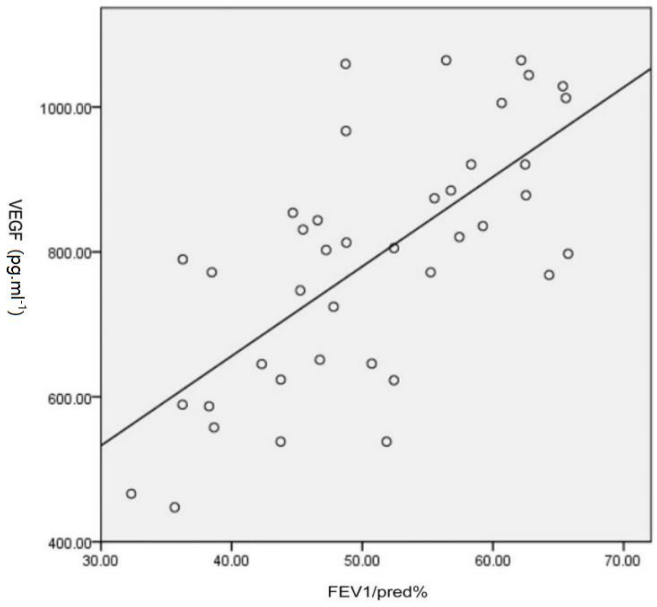

Figure 3. Scatter plot of FEV1/pred% and VEGF concentration in peripheral blood in patients with COPD.

Discussion

COPD is a common lung disease that can mainly affect the health of middle-aged and elderly people[1-2]. Several pathogenic mechanisms have been explained for COPD[3-5]. AECAs are a group of heterogeneous proteins on the surface of endothelial cells that can cause endothelial damage and induce endothelial apoptosis[6,7]. AECA was demonstrated to be involved in emphysema in many studies[11-13]. Another research has indicated an upregulation of AECAs in the serum of COPD patients[14]. Without exposure to cigarette smoke or other toxic gases, COPD patients still suffer from persistent inflammatory responses, indicative of another self-sustained mechanism at work[15]. A recent systematic review also confirmed the potential mechanism of AECAs in developing COPD[16]. The above studies point to the fact that COPD may be an autoimmune disease. Cigarette smoke contains various exogenous compounds, which can be deposited in the lungs, inducing adaptive immune response under specific conditions, including the production of autoantibodies[1,2,17]. One study reported that the positive rate of AECA was 31% in COPD patients vs. 0% in the healthy control group, which implied the correlation between AECA and COPD[18]. The results of our study also found a significant elevation of the AECA concentration of COPD patients than in the healthy controls. This finding supported the previous research arguing for the

significance of AECA in the development of COPD. Besides, we reported a negative correlation between the AECA concentration and FEV_1/pred% in COPD patients. We inferred that AECA might be involved in COPD development and excessive AECA production aggravated COPD. According to the report by Karayama *et al*, the positive rate of AECA was not significantly correlated to patients' age,gender,or clinical manifestations[18], which presents similar results with our present study.

Results from an animal model have shown that the apoptosis of alveolar septal cells may be closely related with emphysema[19]. VEGF have also been demonstrated to be involved in the development of emphysema[11-13]. At the early stage of COPD, pulmonary endothelial cell dysfunction and intimal thickening are accompanied by the downregulation of the genes and proteins of VEGF and VEGF receptors, but accompanied with an increase of the apoptosis of alveolar septal cells[20,21]. Kasahara *et al*.[22] concurred with the above opinion that VEGF downregulation is involved in emphysema by inducing the apoptosis of alveolar septal cells. Another study showed that the VEGF level in COPD patients was negatively correlated with the degree of airflow obstruction[23]. The lower the VEGF level, the more severe the airflow obstruction will be[23]. This study found a significant decrease in the VEGF concentration in COPD patients than in the healthy controls, which confirmed the roles of VEGF in the occurrence and development of COPD as reported. Besides, the VEGF concentration in COPD patients was positively correlated with FEV_1/pred%, so we infer that VEGF may be involved in the development of COPD. Excessive VEGF downregulation may aggravate COPD. Therefore, VEGF may be used as one indicator of COPD severity.

Glucocorticoids (GCs) are a class of steroid hormones secreted by the cortex, and also are suppressors of inflammatory responses[24].GCs are the most common treatment option for inflammatory diseases[24].Studies have shown that GCs can reduce the recurrence and mortality of autoimmune diseases[25]. Previous reports show that GCs can alleviate the symptoms of patients with COPD exacerbation, slowing down the decline in lung function[26,27].As one kind of GCs, methylprednisolone are demonstrated with clinical effectiveness in the clinical trial of COPD treatments[28, 29]. Our study showed that the AECA concentration was significantly lower after methylprednisolone treatment than before in patients with COPD exacerbation. The AECA concentration in methylprednisolone group was also significantly lower than in patients with COPD exacerbation receiving the routine treatment. Besides, the VEGF concentration was significantly elevated in patients with COPD exacerbation after treatment than before. VEGF was also considerably higher in in methylprednisolone group than in patients with COPD exacerbation receiving the routine treatment. Our finding can be concluded that methylprednisolone inhibited AECA expression and promoted VEGF expression. Moreover, methylprednisolone may be conducive to slowing down COPD progression.

Conclusions

AECAs may be involved in the occurrence and development of COPD and related to its severity. Methylprednisolone can help reduce AECA expression while promoting VEGF expression. This mechanism further slows down COPD progression.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by Guizhou Provincial People's Hospital Ethics Committee.

Patient Consent for Publication

Before enrollment, all patients were informed fully and written informed consent was obtained from the patients for the publication of clinical information.

Availability of Data and Material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicting Interest

No conflicts of interest exist.

References

1. Labaki WW, Rosenberg SR. Chronic Obstructive Pulmonary Disease. *Ann Intern Med.* 2020;173(3):ITC17-ITC32.
2. Chronic Obstructive Pulmonary Disease Group of Respiratory Branch of Chinese Medical Association, Guidelines for the diagnosis and treatment of chronic obstructive pulmonary disease (2021 revision). *Chinese Journal of Tuberculosis and Respiratory Medicine.* 2021;44(3):170-205.
3. Kwak N, Lee KH, Woo J, *et al.* Synergistic cycles of protease activity and inflammation via PPARγ degradation in chronic obstructive pulmonary disease. *Exp Mol Med.* 2021;53(5):947-955.
4. Lu Z, Van Eeckhoutte HP, Liu G, *et al*. Necroptosis Signaling Promotes Inflammation, Airway Remodeling, and Emphysema in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med.* 2021;204(6):667-681.
5. MacNee W, Tuder RM. New paradigms in the pathogenesis of chronic obstructive pulmonary disease.Thorac Soc. 2009,6:527-531.
6. Legendre P, Régent A, Thiebault M, *et al*. Anti-endothelial cell antibodies in vasculitis: A systematic review. *Autoimmun Rev.* 2017;

16(2):146-153.

7. Chan TM, Frampton G, Jayne DR, *et al*. Clinical significance of anti-endothelial cell antibodies in systemic vasculitis: a longitudinal study comparing anti-endothelial cell antibodies and anti-neutrophil cytoplasm antibodies. *Am J Kidney Dis*. 1993;22(3):387-92.
8. Domiciano DS, Carvalho JF, Shoenfeld Y. Pathogenic role of anti-endothelial cell antibodies in autoimmune rheumatic diseases. *Lupus.* 2009;18(13):1233-8.
9. Leidinger P, Keller A, Heisel S, *et al*. Novel autoantigens immunogenic in COPD patients. *Respir Res.* 2009;10(1):20.
10. Rovina N, Koutsoukou A, Koulouris NG. Inflammation and immune response in COPD: where do we stand? *Mediators Inflamm*.2013;2013:413-735.
11. Lee SH, Goswami S, Grudo A, *et al*. Antielastin autoimmunity in tobacco smoking-induced emphysema. *Nat Med.* 2007;13(5):567-9.
12. Taraseviciene-Stewart L, Scerbavicius R, Choe KH, *et al*. An animal model of autoimmune emphysema. *Am J Respir Crit Care Med.* 2005;171(7):734-42.
13. Yao H, Zhang L, Zhang C, *et al*. The role of anti-vascular endothelial cell antibodies in rat emphysema and its relationship with methylprednisolone intervention. *China Biochemical Medicine.* 2014:4.
14. Feghali-Bostwick CA, Gadgil AS, Otterbein LE, *et al*. Autoantibodies in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2008;177(2):156-63.
15. Hogg JC, Chu F, Utokaparch S, *et al*. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med.* 2004;350(26):2645-53.
16. Wen L, Krauss-Etschmann S, Petersen F, *et al*. Autoantibodies in Chronic Obstructive Pulmonary Disease. *Front Immunol*. 2018;9:66.
17. Cosio MG, Saetta M, Agusti A. Immunologic aspects of chronic obstructive pulmonary disease. *N Engl J Med*. 2009;360(23):2445-54.
18. Karayama M, Inui N, Suda T, *et al*. Antiendothelial Cell Antibodies in Patients With COPD. *Chest.* 2010;138(6):1303-8.
19. Yan M, Zhang X, Lu P, *et al*. The role of alveolar septal cell apoptosis in autoimmune emphysema in rats. *Chinese Journal of Tuberculosis and Respiratory Diseases.* 2013;5(23): 445-449.
20. Tuder RM, Wood K, Taraseviciene L, *et al*. Cigarette smoke extract decreases the expression of vascular endothelial growth factor by cultured cells and triggers apoptosis of pulmonary endothelial cells. *Chest.* 2000;117(5 Suppl 1):241S-2S.
21. Voelkel N, Taraseviciene-Stewart L. Emphysema: an autoimmune vascular disease? *Proc Am Thorac Soc.* 2005;2(1):23-5.
22. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, *et al*. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest*. 2000;106(11):1311-9.
23. Niu H, Ding Y. Research progress of VEGF in the pathogenesis of COPD. *Hainan Medical Journal.* 2015;26(8):1183-1186.
24. Reichardt SD, Amouret A, Muzzi C, *et al*. The Role of Glucocorticoids in Inflammatory Diseases. *Cells*. 2021;10(11):2921.
25. Tait AS, Butts CL, Sternberg EM. The role of glucocorticoids and progestins in inflammatory, autoimmune, and infectious disease. *J Leukoc Biol.* 2008;84(4):924-31.
26. Leuppi JD, Schuetz P, Bingisser R, *et al*. Short-term vs conventional glucocorticoid therapy in acute exacerbations of chronic obstructive pulmonary disease: the REDUCE randomized clinical trial. *JAMA.* 2013;309(21):2223-31.
27. Ma Z, Zhang W. Short-term versus longer duration of glucocorticoid therapy for exacerbations of chronic obstructive pulmonary disease. *Pulm Pharmacol Ther*. 2016;40:84-90.
28. Sun X, He Z, Zhang J, *et al*. Compare the efficacy of inhaled budesonide and systemic methylprednisolone on systemic inflammation of AECOPD. *Pulm Pharmacol Ther*. 2015;31:111-6.
29. Abubaker J, Zaina F, Siddiqui S, *et al*. Single Intramuscular Methylprednisolone dose in Asthma and Chronic Obstructive Pulmonary Disease Patients on Discharge. *J Pak Med Assoc*. 2019;69(7):991-994.