Research Paper

Influence of Broncho-Vaxom Immunotherapy Combined with Trelegy Ellipta on Blood Eosinophils in Patients with Chronic Obstructive Pulmonary Disease

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To observe effects of Broncho-Vaxom immunotherapy combined with trelegy ellipta on patients with chronic obstructive pulmonary disease and its influence on inflammation-related cells that can be detected in blood routine. 120 patients with stable chronic obstructive pulmonary disease were selected and divided into observation group and control group, with 60 cases in each group. The control group was given Trelegy Ellipta alone and the observation group was given Trelegy Ellipta combined with Broncho-Vaxom immunotherapy. After 3 mo of treatment, the blood routine, immune function, lung function and clinical scores were compared between the two groups and the adverse effects of drugs were recorded. After 12 mo of follow-up, the number of acute exacerbations in patients was statistically analyzed. During treatment, the total incidence rate of adverse reactions showed no statistical difference between the two groups of patients ($p>0.05$). Within 12 mo of follow-up, the number of acute exacerbations was (0.41±0.12) times in the observation group, which was lower than (0.61±0.25) times in control group ($p<0.05$). The application of Broncho-Vaxom immunotherapy combined with Trelegy Ellipta on patients with stable chronic obstructive pulmonary disease can lower the level of eosinophil in peripheral blood, regulate the immune function, improve the lung function, enhance the quality of their life and reduce the number of acute exacerbations, as well as with good safety.

Key words: Chronic obstructive pulmonary disease, immunotherapy, bacterial lysate, fluticasone furoate, umeclidinium bromide, vilanterol trifenatate, inhalation, eosinophil

Chronic Obstructive Pulmonary Disease (COPD) is a chronic airway disease characterized by incompletely reversible limitation of airflow, which has a long course and shows progressive development. The lung function of COPD patients will decline continuously due to exacerbation and recurrence of the disease if there is no long-term and standardized treatment[1]. For patients with stable COPD, the main goal of treatment is to relieve symptoms, reduce the times of acute episodes, and delay the progression of the disease[2]. Trelegy Ellipta powder for inhalation is a compound preparation with three active ingredients includes fluticasone furoate, umeclidinium and vilanterol triphenylacetate which have properties of anti-inflammatory, dilating bronchus, relieving smooth muscle spasm, etc. The drug has been used more and more in the treatment of COPD since it was launched in China in November 2019[3]. In recent years, many studies have pointed out that airway mucosal immune dysfunction plays an important role in the pathological process of COPD. Therefore, the immune reconstitution and repair of the airway through immune regulation has attracted an increasing attention in the treatment of COPD patients[4]. As an immunostimulant, bacterial lysate (Broncho-Vaxom) can reinforce the immune function of the respiratory tract by activating the specific mucosal immune response. At present, Broncho-Vaxom has certain applications in the prevention and treatment of recurrent respiratory infections and chronic bronchitis[5]. In this study, Broncho-Vaxom combined with Trelegy Ellipta was used in the treatment of patients with stable COPD, aiming to observe the clinical effects and influence on the patients' inflammation-related cells in blood routine.

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MATERIALS AND METHODS

General information:

A total of 120 COPD patients admitted to the respiratory clinic from November 2019 to September 2020 were selected. The inclusion criteria includes those who met the diagnostic criteria for stable COPD according to the "Guidelines for the Diagnosis and Treatment of Chronic Obstructive Pulmonary Disease (2013 Revised Edition)"[6]; 40~75 y old; normal mental state and cognition; no previous drug treatment which would be used in this study; available follow-up contact information; those who signed informed consent. Exclusion criteria includes acute exacerbation of COPD; those who had severe bronchial asthma, acute respiratory failure and other serious respiratory diseases; those with complications including heart disease, liver and kidney insufficiency, acquired immune deficiency syndrome, blood system disease, infectious disease and other serious basic diseases; Use of immunomodulators in the past 3 mo; history of allergy to drugs used in this research; pregnant or lactating women. According to the random envelope method, the subjects were divided into an observation group and a control group with 60 cases in each group. The observation group has 36 males and 24 females; age is 42~75 (63.15±9.84) y old, with a course of COPD of 3~13 (6.78±2.95) y; the number of acute exacerbations in the past year is 1~6 (2.25±0.58) times; the COPD patients self-Assessment Test (CAT) score is 11~28 (20.96±5.52) at the time of enrollment. The control group has 39 males and 21 females; age is 40~73 (62.08±8.46) y; COPD course is 2~12 (6.41±2.67) y; the times of acute exacerbations are 1~6 (2.18±0.49) in the past year; CAT score is 11~26 (20.28±5.16). There was no statistically significant difference in general information including gender, age and course of COPD between the two groups of patients ($p>0.05$), which makes them comparable.

Methods:

Both groups of patients received general treatment and health guidance, mainly including smoking and alcohol cessation, long-term family oxygen therapy (oxygen flow was 1.0~2.0 l/min, smoking time was 10~15 h) and rehabilitation therapy (breathing exercises, systemic exercise, nutritional support etc.,) and supportive treatments to resolve phlegm and relieve cough based on the patient's condition. The control group was treated with Trelegy Ellipta (Manufacturer: Glaxo Operations UK Ltd) and the National Medicine Standard is H20190055, with 30 inhalations/box, each inhalation contains 100 μg of fluticasone furoate, 62.5 μg of umeclidinium and 25 μg of vilanterol triphenylacetate. The drug was given by inhalation with one inhalation per time and once per day, the patients were instructed to rinse their mouths with water after inhalation. The observation group was given Trelegy Ellipta combined with Broncho-Vaxom (Manufacturer: OM Pharma SA, the National Medicine Standard is SJ20150042, specification is 7 mg×10 capsules), who took one capsule on an empty stomach every morning for 10 consecutive days and stopped taking medication for the next 20 d in 1 mo. Each course of treatment was 3 mo. Both groups of patients were rechecked with relevant clinical indicators in the outpatient clinic after 3 mo of treatment.

Observation indicators:

Blood routine: 5 ml of cubital venous blood from fasting patients was collected before and after treatment and the laboratory tested the inflammation-related cells in the blood routine, including White Blood Cell (WBC) count, Eosinophil (EOS) count and Neutrophil-Lymphocyte Ratio (NLR).

Immune function: 5 ml of cubital venous blood from fasting patients was collected and separated the serum (3000 r/min, 10 min) and detected the levels of Immunoglobulin A (IgA), Immunoglobulin G (IgG) and Immunoglobulin M (IgM) by scattering turbidimetric method. The detection instrument is IMMAGE800 specific protein analysis system (Beckman Coulter, USA).

Pulmonary function: The MasterScreen PFT system pulmonary function meter (Jerger, Germany) was used to detect the patients pulmonary function indicators before and after treatment, including the Forced Expiratory Volume in the first second (FEV1) and the percentage of FEV1 occupied in Forced Expiratory Vital Capacity (FVC) [FEV1/FVC %] and Peak Expiratory Flow rate (PEF).

Clinical scoring: CAT score and St. George's Respiratory Questionnaire (SGRQ)[7] were used to evaluate the severity of the patient's condition and quality of life before and after treatment. The CAT score has a total of 8 questions and each question is graded from 1 to 5. The higher the score is the more serious the condition will be. SGRQ includes symptoms, activity restrictions and daily life impacts, which uses the weighted-average method to calculate the total score. The score ranges from 0 to 100 points. The higher the score is, the worse the quality of life will be.

Adverse reactions: We recorded the occurrence of

adverse reactions of drugs during the treatment of patients.

Follow-up: By telephone or outpatient clinic, with monthly follow-up for the first 3 mo and once every 3 mo in the later period, and the times of acute exacerbations (that is, the patient is admitted to the hospital again for diagnosis and treatment due to acute exacerbations) were collected.

Statistical analysis:

Statistical Package for the Social Sciences (SPSS) 22.0 software was used and the measurement data were described with "$\bar{x}\pm s$". The independent sample t-test was used for comparison between two groups and the paired sample t-test was used for comparison between two groups before and after the treatment. The count data are listed as examples (%), and χ^2 test or Fisher's exact probability test was performed for analysis, $p<0.05$ indicates that the difference is statistically significant.

RESULTS AND DISCUSSION

In the observation group, 2 patients were lost to follow-up after leaving the hospital, with a total of 58 effective cases and a follow-up rate of 96.67 %; in the control group, four patients were lost to follow-up after leaving the hospital, with a total of 56 effective cases and a follow-up rate of 93.33 %.

There was no statistically significant difference in blood, WBC, EOS and NLR between the two groups of patients before treatment ($p>0.05$); after treatment, WBC, EOS and NLR in both groups of patients decreased compared with before treatment ($p<0.05$) and the EOS and NLR of the observation group were lower than those of the control group ($p<0.05$) as shown in Table 1.

Before treatment, there was no significant difference in serum IgA, IgG and IgM between the two groups of patients ($p>0.05$); after treatment, the levels of IgA, IgG and IgM in the two groups of patients were higher than before ($p<0.05$). The levels of IgG and IgM in the observation group were higher than those in the control group ($p<0.05$) as shown in Table 2.

Before treatment, there was no significant difference in FEV1, FEV1/FVC and PEF between the two groups of patients ($p>0.05$); after treatment, the FEV1, FEV1/FVC and PEF of the two groups were all higher than before treatment ($p<0.05$) and FEV1, FEV1/FVC, PEF in the observation group were higher than those in the control group ($p<0.05$) as shown in Table 3.

Before treatment, there was no statistically significant difference in CAT and SGRQ scores between the two groups of patients ($p>0.05$); after treatment, the CAT and SGRQ scores of the two groups were lower than before treatment ($p<0.05$) and the CAT and SGRQ scores in the observation group were lower than those in the control group ($p<0.05$) as shown in Table 4.

During the treatment, there was no significant difference in the total incidence of adverse effects between the two groups of patients ($p>0.05$) and there were no adverse reactions that seriously affected the treatment as shown in Table 5.

TABLE 1: COMPARISON OF INFLAMMATION RELATED CELLS IN BLOOD ROUTINE IN THE TWO GROUPS OF PATIENTS BEFORE AND AFTER TREATMENT ($\bar{x}\pm s$)

Group	n	WBC ($\times10^9$/l)		EOS ($\times10^9$/l)		NLR	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation	58	8.95±1.64	7.21±1.26[a]	0.42±0.15	0.17±0.06[a]	5.36±1.92	2.07±0.45[a]
Control	56	8.87±1.43	7.44±1.05[a]	0.40±0.16	0.28±0.11[a]	5.25±1.81	2.91±0.82[a]
t		0.277	1.057	0.689	6.659	0.315	6.812
p		0.782	0.293	0.492	<0.001	0.754	<0.001

Note: Compared with the variable before treatment, [a]$p<0.05$; WBC: White Blood Cells; EOS: Eosinophils and NLR: Neutrophil-Lymphocyte Ratio

TABLE 2: COMPARISON OF IMMUNOGLOBULINS BETWEEN THE TWO GROUPS OF PATIENTS BEFORE AND AFTER TREATMENT (x̄±s)

Group	n	IgA		IgG		IgM	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation	58	1.62±0.79	2.77±0.83[a]	9.24±2.48	13.21±2.28[a]	0.67±0.25	1.68±0.63[a]
Control	56	1.57±0.65	2.58±0.76[a]	9.37±2.59	11.75±2.83[a]	0.71±0.29	1.34±0.48[a]
t		0.368	1.273	0.274	3.038	0.79	3.233
p		0.713	0.206	0.785	0.003	0.431	0.002

Note: Compared with the variable before treatment, [a]p<0.05; IgA: Immunoglobulin A; IgG: Immunoglobulin G and IgM: Immunoglobulin M

TABLE 3: COMPARISON OF LUNG FUNCTION INDICATORS BEFORE AND AFTER TREATMENT BETWEEN THE TWO GROUPS (x̄±s)

Group	n	FEV1 (l)		FEV1/FVC (%)		PEF (l/s)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Observation	58	1.18±0.42	1.84±0.55[a]	51.82±7.24	64.28±6.46[a]	5.27±1.52	6.84±1.79[a]
Control	56	1.23±0.39	1.58±0.47[a]	50.95±6.28	58.77±6.92[a]	5.46±1.68	6.13±1.44[a]
t		0.658	2.709	0.684	4.396	0.634	2.427
p		0.512	0.008	0.495	<0.001	0.528	0.017

Note: Compared with the variable before treatment, [a]p<0.05; FEV1: Forced Expiratory Volume in the first second, FVC: Forced Expiratory Vital Capacity and PEF: Peak Expiratory Flow rate

TABLE 4: COMPARISON OF RELATED CLINICAL SCORES BETWEEN THE TWO GROUPS BEFORE AND AFTER TREATMENT (x̄±s, SCORES)

Group	n	CAT		SGRQ	
		Before treatment	After treatment	Before treatment	After treatment
Observation	58	20.96±5.52	9.84±2.61[a]	50.32±14.81	33.28±7.46[a]
Control	56	20.28±5.16	13.65±3.35[a]	49.24±12.35	40.51±10.04[a]
t		0.68	6.787	0.422	4.375
p		0.499	<0.001	0.674	<0.001

Note: Compared with the variable before treatment, [a]p<0.05; SGRQ: St. George's Respiratory Questionnaire and CAT: COPD patients self-Assessment Test

TABLE 5: COMPARISON OF THE INCIDENCE OF ADVERSE EFFECTS BETWEEN THE TWO GROUPS OF PATIENTS [n (%)]

Group	n	Respiratory tract discomfort	Headache	Gastrointestinal disorders	Tachycardia	Rash	Total incidence
Observation	58	3 (5.17)	2 (.45)	1 (1.72)	1 (1.72)	1 (1.72)	8 (13.79)
Control	56	1 (1.79)	3 (5.36)	1 (1.79)	2 (3.57)	0 (0.00)	7 (12.50)
χ^2		--	--	--	--	--	0.042
p/fisher p		0.619	0.676	1	0.615	1	0.838

Note: Compared with the variable before treatment, p<0.05

Within 12 mo of follow-up, the times of acute exacerbations in the observation group was 0~2, with an average of 0.41±0.12 and the frequency in the control group was 0~4, with an average of 0.61±0.25 times. The number of acute exacerbations in the observation group were significantly lower than those in the control group ($t=5.475$, $p<0.001$).

At present, the cause of COPD remains unclear. It is generally acknowledged that long-term exposure to environmental factors (including air pollution, smoking, chemical substance inhalation, etc.,) interacting with body factors (such as genetic factors, pulmonary dysplasia, increased airway reactivity, etc.,) leads to COPD[8]. Drug therapy occupies a dominant position in COPD disease control, which is used to relieve symptoms, improve lung function, reduce the extent and frequency of acute exacerbation, thereby delaying the progression of the disease and improving the patients' quality of life. Therefore, choosing safe and effective drugs for COPD treatment is attracting increasing attention in clinical research.

Trelegy Ellipta is a triple preparation composed of fluticasone furoate, umeclidinium and vilanterol triphenylacetate. Fluticasone furoate is a new synthetic glucocorticoid trifluoride. Because of its unique 17-α furoate structure, fluticasone furoate has higher glucocorticoid receptor binding capacity and stronger anti-inflammatory activity compared with dexamethasone and fluticasone propionate. Besides, unlike other glucocorticoids, fluticasone furoate is beneficial for maintaining the epithelial cell integrity of the respiratory tract[9]. Umeclidinium is a new long-acting anticholinergic drug with high selectivity for M3 receptors, which can compete with acetylcholine for M receptors in a dose-dependent manner and further inhibit the excitement of the vagus nerve, reduce glandular secretion and dilate the bronchus[10]. Vilanterol is a highly selective and long acting beta-2 (β_2) receptor agonist, which can promote the conversion of adenosine triphosphate to cyclic adenosine monophosphate by activating intracellular adenylate cyclase, thereby activating protein kinases and inhibiting calcium dependence K^+ channel phosphorylation or light chain kinase activity. The downstream effect is smooth muscle relaxation, cilia movement and reduction of inflammatory mediators[11]. Previous studies have proved that the use of triple preparation including fluticasone furoate/umeclidinium/vilanterol has positive effects in reducing the symptoms of COPD patients and improving their quality of life[12]. After the control group of this study was treated with Trelegy Ellipta, the patients' lung function, CAT, SGRQ scores, etc., have improved and there were no serious adverse effects, which indicated that Trelegy Ellipta is safe and effective for the maintenance treatment of COPD.

Broncho-Vaxom is an oral immune stimulant composed of 8 common respiratory tract pathogens (*Streptococcus pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pyogenes, Klebsiella Bacteria, Streptococcus viridans* and *Neisseria catarrhalis*) are separated, lysed and purified to obtain their glycoproteins, which can activate the body's humoral immunity and cellular immunity through specific and non-specific ways of stimulation, thus exerting the effect of immune protection[13]. Animal experiments pointed out[14,15] that Broncho-Vaxom can stimulate the activity of macrophages and B lymphocytes, thereby promoting the secretion of immunoglobulins in the respiratory mucosa and enhancing the resistance to infection in experimental animals. Some studies have also shown that Broncho-Vaxom can reduce airway remodeling in guinea pigs with bronchial asthma by regulating the balance of transforming growth factor-β_1 and Smad7 protein expression. Broncho-Vaxom has been used in the immunotherapy of children with repeated respiratory tract infections in the past, and it is believed to be able to effectively regulate the immune function of children and reduce the occurrence of respiratory tract infections[16].

Airway inflammation occupies an important position in the onset and acute exacerbation of COPD. EOS, as an essential type of cell in the inflammatory response, can promote the development of inflammation and mediate tissue damage by releasing particle contents, which are closely related to the acute exacerbation of COPD[17]. NLR is an indicator that reflects the balance of neutrophils and lymphocytes. For COPD patients, due to decreased immune function or the stress response state during an acute exacerbation, the count of lymphocytes in peripheral blood can be reduced accordingly. At the same time, the number of neutrophils in peripheral blood will increase due to bacterial infection, inflammatory factors and other stimuli. Therefore, the increase of NLR can reflect the inflammatory state of COPD patients and the risk of acute exacerbation[18]. The results of this article show that the EOS and NLR of the observation group treated with Trelegy Ellipta combined with Broncho-Vaxom were lower than those of the control group after treatment, while the level of serum IgG and IgM were higher than

those of the control group. Similar to previous reports[19], the mechanism may be related to the ability of Broncho-Vaxom to regulate the body's humoral and cellular immunity, establish an immune barrier of the respiratory tract and reduce airway inflammation and damage. This study found that FEV1, FEV1/FVC and PEF of the observation group were higher than those of the control group after treatment, while the CAT and SGRQ scores were lower than those of the control group and the number of acute exacerbations within 12 mo was significantly lower than that of the control group. These results indicate that Broncho-Vaxom combined therapy can improve lung function, reduce the severity of illness, improve the quality of life and reduce the frequency of acute exacerbations in COPD patients, which may be related to the enhancement of the patients' immune function and the reduction of airway inflammation. Studies by Zeng *et al.*[20] have shown that Broncho-Vaxom can regulate the immune function, reduce the number of acute exacerbations, improve the quality of life, and delay the decline of lung function in COPD patients, which supports the results of our study. In addition, only minor adverse effects were occasionally observed during the treatment in this study and there was no statistically significant difference in the total incidence of adverse reactions between the two groups of patients.

Based on the results, we conclude that combining Broncho-Vaxom immunotherapy with Trelegy Ellipta can effectively down-regulate the count of EOS in the peripheral blood of COPD patients, which can also regulate immune function and improve the lung function of COPD patients. Besides, Broncho-Vaxom combined with Trelegy Ellipta can reduce the frequency of acute exacerbations and improve the patients' quality of life. We also proved that this therapy has reliable safety and is feasible in clinical application.

Author's contributions:

Bo Li and Fan Yang have contributed equally to this work.

Conflict of interests:

The authors declared no conflicts of interest.

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