Analysis of curative effect of adjuvant therapy with bronchoalveolar lavage on COPD patients complicated with pneumonia

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Abstract. Clinical effect of adjuvant therapy with bronchoalveolar lavage on chronic obstructive pulmonary disease (COPD) patients complicated with pneumonia and its influence on the expression levels of inflammatory factors were studied. One hundred and twenty mild-moderate COPD patients complicated with pneumonia treated in the Department of Respiratory Medicine, The Sixth People's Hospital of Nantong from February 2016 to February 2017 were selected and randomly divided into three groups: One-time lavage group (n=40), two-time lavage group (n=40) and control group (n=40). Fasting peripheral blood was collected from all the patients in the morning. The lung function and blood gas analyses, and the detection of peripheral white blood cells (WBC), procalcitonin (PCT) and C-reactive protein (CRP) were performed. Moreover, the messenger RNA (mRNA) levels of interleukin-6 (IL-6), IL-8, tumor necrosis factor-α (TNF-α) and leukotriene B4 (LTB4) in lavage fluid were detected via reverse transcription-quantitative polymerase chain reaction. The lung functions of patients in the two-time lavage group were significantly improved compared with that in the one-time lavage group ($p<0.01$). pH and PaO_2 in two-time lavage group were higher than those in the one-time lavage group ($p<0.01$). Peripheral WBC, PCT and CRP levels in the two-time lavage group were lower than those in the one-time lavage group ($p<0.05$). The mRNA levels of IL-6, IL-8, TNF-α and LTB4 in lavage fluid in two-time lavage group were lower than those in one-time lavage group ($p<0.01$). IL-6, IL-8, TNF-α and LTB4 expression levels in lavage fluid in two-time lavage group were lower than those in one-time lavage group ($p<0.01$). In conclusion, the adjuvant therapy with bronchoalveolar lavage improves the therapeutic effect on COPD patients complicated with pneumonia, which can significantly reduce the expression levels of inflammatory factors, and facilitate the control of pulmonary infection and recovery of lung function.

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic airflow obstruction disease, which, as one of the common chronic diseases in the respiratory system, has high morbidity and mortality rates (1,2). The incidence of COPD is progressive, ultimately leading to the loss of lung function and damage to lung tissues, seriously affecting the health and life quality of patients (3). Clinically, pneumonia refers to pulmonary parenchymal and interstitial infectious inflammation, even spreading across the whole lung, caused by a variety of factors, among which bacterial infection is the most common. COPD often leads to the occurrence of pneumonia in the long-term chronic development (4).

At present, the conventional phlegm-dispelling methods of COPD include anti-inflammatory, phlegm-eliminating and antispasmodic drug therapies, and physical therapies of aerosol inhalation and high-frequency chest wall oscillation. Although these methods can alleviate the symptoms to some extent, they cannot fully drain the sputum and the curative effects are slow (5). Moreover, the accumulation of inflammatory mucus exudate in small airway lumen inducing the formation of inflammatory immune cells in lymphoid follicles. As a result, the infiltration of a large number of immune cells in the airway wall aggravate COPD complicated with pneumonia (5). Therefore, the clearance of airway secretions is critical in the treatment of COPD. Einarsson *et al* (6) studied and found that the bronchoalveolar lavage using bronchoscope for COPD patients complicated with respiratory failure can effectively improve the oxygenation of patients, and benefit treatment of the disease. Górka *et al* (7) found that bronchoalveolar lavage can remove the airway secretions, effectively alleviate the airway obstruction, and shorten the hospitalization time of patients with pulmonary infection. Bronchoalveolar lavage is widely used in clinical practice, especially in patients with airway

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mucus hypersecretion, which can quickly and effectively remove the airway mucus, improve the airway ventilation, make the inhaled drugs directly contact with airway wall, and reduce the airway inflammation, thus improving the clinical effect on COPD. Therefore, the role of bronchoalveolar lavage in the treatment of COPD has attracted increasingly more attention (8). Whether bronchoalveolar lavage is necessary in the treatment of patients with mild-moderate COPD remains to be determined. Based on the above research progress, the clinical effect of adjuvant therapy with bronchoalveolar lavage on COPD patients complicated with pneumonia and its influences on the expression levels of inflammatory factors were analyzed in this study, so as to demonstrate the effectiveness and safety of bronchoalveolar lavage.

Materials and methods

Subjects of study. In total, 120 mild-moderate COPD patients complicated with pneumonia treated in the Department of Respiratory Medicine, The Sixth People's Hospital of Nantong (Nantong, China) from February 2016 to February 2017 were selected and screened according to the Guidelines on Diagnosis of Chronic Obstructive Pulmonary Disease of Chinese Medical Association. The ratio of forced expiratory volume in 1 sec to forced vital capacity (FEV1/FVC) <70% after inhalation of bronchodilators indicated the persistent airflow obstruction. Combined with the chief complaint, patients with dyspnea complicated with cough and expectoration can be diagnosed with COPD complicated with pneumonia. Exclusion criteria for the study were: Patients with respiratory arrest, significant agitation, who did not cooperate in the examination, without cough reflex, with hemodynamic instability, severe arrhythmia, liver or kidney failure or other wasting diseases. The patients meeting the requirements were randomly divided into three groups, one-time lavage group (n=40), two-time lavage group (n=40) and control group (n=40). Patients in the one-time lavage group were treated with bronchoalveolar lavage once at 8 days after admission and the alveolar lavage fluid was retained. Patients in the two-time lavage group were treated with bronchoalveolar lavage at 3 and 8 days after admission and the alveolar lavage fluid was retained. Patients in control group did not receive bronchoalveolar lavage. The same basic anti-infective therapy was applied to all the patients in the three groups. There were no statistically significant differences in age, sex and course of disease among groups. Clinical data and pathological data during hospitalization were retained. The study was approved by the Ethics Committee of The Sixth People's Hospital of Nantong and informed consents were signed by the patients and/or guardians.

Treatment methods

Basic therapy. The patients selected received the same basic therapy, including the application of broad-spectrum antibiotics, bronchodilators, expectorants and glucocorticoids.

Bronchoalveolar lavage. Lavage was performed at the bedside for patients using the Olympus BF-P60 portable fiber bronchoscope. Patients received all examinations after admission, and intramuscular injection of 654-2 before lavage. Lidocaine (2%) was used for local aerosol anesthesia, and a total of 100 ml normal saline at approximately 30˚C was used as lavage fluid. After lavage, the lavage fluid and sputum were sucked out, and the nursing staff slapped patients' back at the bedside to help sputum flow out better.

Determination of lung function. The lung function of patients in each group was detected by the professional physician in the Department of Respiratory Medicine using the lung function apparatus (Jaeger Master Diffusion; Jaeger, Hoechberg, Germany). With a straight back, the patients wore the nasal splint and looked ahead, and the mouthpiece was placed in the patients' mouth without air leakage. The ratio of forced expiratory volume in 1 sec to forced vital capacity (FEV1/FVC), peak expiratory flow (PEF), 25% PEF (PEF25) and peak expiratory flow rate (PEFR) were recorded.

Arterial blood gas and blood biochemical analyses. Patients did not stop or stopped oxygen inhalation for >30 min when arterial blood gas analysis was performed. Arterial blood (2 ml) was collected from the radial artery of all the patients for immediate detection. The arterial blood gas indexes [pH, partial pressure of oxygen (PaO_2) and partial pressure of carbon dioxide ($PaCO_2$)] and blood biochemical indexes [peripheral white blood cells (WBC), procalcitonin (PCT) and C-reactive protein (CRP)] of each patient were recorded.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). TRIzol reagent (TRIzol kit; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was added into the lavage fluid of patients in each group at a volume ratio of 1:1, mixed, added with 1 ml chloroform, and let stand for 10 min, followed by centrifugation at 9,800 x g at 4˚C for 10 min. After the supernatant was absorbed, an equal volume of isopropyl alcohol was added and mixed, followed by centrifugation at 9,800 x g at 4˚C for 10 min. The supernatant was discarded, and 75% ethanol was added to fully wash the sediment. After the supernatant was discarded, the mixture was dried at room temperature for approximately 5 min, and then the sediment was dissolved using 50 μ diethylpyrocarbonate (DEPC) water; thus, the RNA was obtained. The purity and concentration of RNA obtained were detected via agarose gel and optical density (OD) value, respectively. It was found that the RNA above met the requirements of reverse transcription. The complementary DNA (cDNA) strands were obtained after reverse transcription using the reverse transcription kit strictly according to the above requirements. With cDNA strands as the template, primers, Taq polymerase, Taq buffer, deoxy-ribonucleoside triphosphate (dNTP) mixture and double-distilled water (ddH_2O) were added for PCR amplification on the PCR instrument. Finally, the product was placed on the quantitative PCR instrument to detect the mRNA expression of target genes, and the mRNA expression levels of interleukin-6 (IL-6), IL-8, tumor necrosis factor-α (TNF-α) and leukotriene B4 (LTB4) were also calculated.

Detection of inflammatory factor levels via enzyme-linked immunosorbent assay (ELISA). The concentrations of IL-6, IL-8, TNF-α and LTB4 in lavage fluid of patients in each group were detected using the corresponding ELISA kits, and the standard curves prepared via the standard sample were used as the quantitative criteria. In accordance with the steps of the ELISA kit, (IL-6, IL-8 and TNF-α; cat. nos. KE00007, KE00006 and

Table I. General data of patients in each group (mean ± SD).

Groups	Sex (male/female)	Age (years)	Heart rate (time/min)	Respiratory rate (time/min)	COPD course (mild/moderate)
One-time lavage	(19/21)	32.7±12.4	83.2±11.9	22.5±4.4	(26/14)
Two-time lavage	(18/22)	31.5±18.7	85.6±9.1	23.6±5.2	(25/15)
Control	(20/20)	30.9±8.5	82.1±7.5	23.4±3.1	(26/14)
P-value	>0.05	>0.05	>0.05	>0.05	>0.05
t-test	0.832	0.653	0.721	0.855	0.639

COPD, chronic obstructive pulmonary disease.

Table II. Lung function monitoring of patients in each group (mean ± SD).

Groups	FEV1/FVC (%)	PEF	PEF25	PEFR (liter/sec)
One-time lavage				
Before treatment	58.67±12.4	65.22±7.3	55.12±10.3	2.5±0.4
12 days after treatment	78.59±7.3[b,c]	81.27±6.9[b,c]	68.33±5.2[b,c]	3.2±0.6[b,c]
Two-time lavage				
Before treatment	55.12±8.3	63.15±8.1	53.26±7.5	2.2±0.6
12 days after treatment	86.55±7.2[b,d]	93.22±5.1[b,d]	76.39±2.9[b,d]	3.8±0.5[b,d]
Control				
Before treatment	56.55±9.4	61.92±8.5	52.17±6.1	2.6±0.3
12 days after treatment	69.25±4.4[b]	72.21±3.6[b]	62.32±5.0[b]	2.9±0.5[a]

Compared with that before treatment, [a]p<0.05, [b]p<0.01; compared with control group, [c]p<0.05, [d]p<0.01. FVC, forced vital capacity; PEF, peak expiratory flow; PEFR, peak expiratory flow rate.

KE00068, respectively; all obtained from ProteinTech Group, Inc., Chicago, IL, USA), the lavage fluid (diluted at 1:10) of each group was added into the sample well and the plate was sealed using the sealing membrane, followed by incubation for 60 min. Then the corresponding biotin-labeled antibody was added for incubation at 37˚C for 60 min. The plate was then washed with cleaning solution and added with 100 μl avidin-peroxidase complex for reaction at 37˚C for 30 min. The liquid waste was discarded, and the plate was washed again with cleaning solution. Finally, the stop buffer was added to terminate the reaction; the OD value of samples in each group was measured, and the serum IL-6, IL-8, TNF-α and LTB4 levels in each group were calculated through the standard curve.

Statistical analysis. Data in this study were presented as mean ± standard deviation and analyzed using Statistical Product and Service Solutions (SPSS) 19.0 statistical software (SPSS, Inc., Chicago, IL, USA). The t-test was used for the intergroup comparison, and Chi-square test was used for enumeration data. Analysis of variance and SNK post hoc test were used for the comparison among groups. P<0.05 was considered to indicate a statistically significant difference.

Results

General data. A total of 120 mild-moderate COPD patients complicated with pneumonia were selected and randomly divided into the one-time lavage, two-time lavage and control groups. There were 19 males and 21 females aged 32.7±12.4 years in the one-time lavage group, 18 males and 22 females aged 31.5±18.7 years in the two-time lavage group, and 20 males and 20 females aged 30.9±8.5 years in the control group. The subjects underwent examinations within 24 h after admission. The results showed that there were no statistically significant differences in sex, age, heart rate, respiratory rate and course of COPD among groups (p>0.05) (Table I).

Lung function monitoring. The lung function of patients in each group was monitored by doctors using the lung function apparatus; the lung functions before and after treatment were recorded. The results showed that after treatment for 12 days, FEV1/FVC, PEF, PEF25 and PEFR of patients in each group were increased (p<0.05); compared with those in the control group, FEV1/FVC, PEF, PEF25 and PEFR of patients in the one-time and two-time lavage groups were significantly increased (p<0.01). These indexes were higher in the two-time lavage group than those in the one-time lavage group (p<0.01) (Table II).

Blood gas analysis. Arterial blood gas analysis was performed for the patients in each group. The results revealed that after treatment for 12 days, PaO_2 of patients in each group was obviously increased, but pH and $PaCO_2$ were obviously decreased (p<0.05). Compared with those in control group,

Table III. Blood gas analysis of patients in each group (mean ± SD).

Groups	pH	PaO_2 (mmHg)	$PaCO_2$ (mmHg)
One-time lavage			
Before treatment	7.29±0.04	73.66±27.5	53.05±60.3
12 days after treatment	7.21±0.03[a,b]	86.82±26.3[a,b]	46.33±5.2[a,b]
Two-time lavage			
Before treatment	7.27±0.03	76.76±25.6	55.36±8.2
12 days after treatment	7.13±0.02[a,c]	92.21±20.3[a,c]	42.19±6.7[a,c]
Control			
Before treatment	7.30±0.04	75.36±28.2	54.25±5.6
12 days after treatment	7.25±0.04[a]	82.17±23.5[a]	49.11±5.3[a]

Compared with that before treatment, [a]p<0.01; compared with control group, [b]p<0.05, [c]p<0.01. PaO_2, partial pressure of oxygen; $PaCO_2$, partial pressure of carbon dioxide.

Table IV. Biochemical index detection (mean ± SD).

Groups	WBC ($x10^9$)	PCT (pg/ml)	CRP (mg/l)
One-time lavage			
Before treatment	13.52±5.24	1.66±0.95	33.9±6.7
12 days after treatment	5.55±1.23[a,b]	0.52±0.13[a,b]	11.5±3.2[a,b]
Two-time lavage			
Before treatment	13.26±4.85	1.68±1.06	39.5±7.3
12 days after treatment	4.36±1.63[a,c]	0.28±0.09[a,c]	9.2±1.6[a,c]
Control			
Before treatment	13.37±4.96	1.62±0.82	36.6±5.9
12 days after treatment	6.97±2.65[a]	0.87±0.12[a]	16.1±2.3[a]

Compared with that before treatment, [a]p<0.01; compared with control group, [b]p<0.05, [c]p<0.01. WBC, white blood cells; PCT, procalcitonin; CRP, C-reactive protein.

PaO_2 in one-time lavage group and two-time lavage group were significantly increased, but pH and $PaCO_2$ were significantly decreased (p<0.01). In addition, compared with those in one-time lavage group, PaO_2 in two-time lavage group was significantly increased, but pH and $PaCO_2$ were significantly decreased (p<0.01) (Table III).

Biochemical index detection. Each biochemical index in peripheral blood of patients was detected before treatment and at 12 days after treatment. The results revealed that peripheral WBC, PCT and CRP levels of patients in each group after treatment were significantly decreased compared with those before treatment (p<0.05). After treatment for 12 days, peripheral WBC, PCT and CRP levels in one-time lavage group and two-time lavage group were significantly decreased compared with those in the control group (p<0.01); the levels in two-time lavage group were lower than those in one-time lavage group (p<0.01) (Table IV).

Detection of messenger RNA (mRNA) expression levels of inflammatory factors. The mRNA expression levels of IL-6, IL-8, TNF-α and LTB4 in lavage fluid of patients in each group were detected via RT-qPCR. The results revealed that the mRNA levels of IL-6, IL-8, TNF-α and LTB4 in lavage fluid of patients in each group were significantly decreased after treatment (p<0.05). After treatment for 12 days, the mRNA levels of IL-6, IL-8, TNF-α and LTB4 in lavage fluid in the one-time lavage and two-time lavage groups were obviously decreased compared with those in control group (p<0.01); the levels in lavage fluid in two-time lavage group were lower than those in one-time lavage group (p<0.01) (Table V).

Detection of expression levels of inflammatory factors. The levels of IL-6, IL-8, TNF-α and LTB4 in lavage fluid of patients in each group were detected using the ELISA kit. The results showed that the levels of IL-6, IL-8, TNF-α and LTB4 in lavage fluid of patients in each group were significantly decreased after treatment for 12 days (p<0.05). Compared with those in control group, the levels of IL-6, IL-8, TNF-α and LTB4 in lavage fluid in one-time lavage group and two-time lavage group were obviously decreased (p<0.01); the levels in lavage fluid in two-time lavage group were lower than those in one-time lavage group (p<0.01) (Table VI).

Discussion

China has a high incidence of COPD, and with an increasing population, environment deterioration and prominent aging problem, the number of COPD patients complicated with pneumonia has also shown a growing trend (9). Alveolar lavage using antibiotics based on the bronchoscope technique has been widely applied in clinic to remove the lung mucus and control inflammation, which can effectively increase the treatment effective rate of COPD patients complicated with pneumonia, and reduce the inflammatory response (10). Fortún *et al* (11) treated the COPD patients complicated with type II respiratory failure using alveolar lavage combined with mechanical ventilation, and the results showed that CRP declined and blood gas analysis were significantly improved. Gasiuniene *et al* (12) studied the curative effect of bronchoalveolar lavage on COPD patients complicated with pulmonary infection, and found that sputum suction and lavage via fiber bronchoscope can improve the symptoms, increase the cure rate and shorten the length of hospital stay.

In this study, the clinical effect of adjuvant therapy with bronchoalveolar lavage on COPD patients complicated with pneumonia was investigated. The results showed that bronchoalveolar lavage could effectively improve the lung function and increase the pulmonary ventilation of COPD patients complicated with pneumonia. Lung function is a necessary condition for the diagnosis of COPD, in which FEV1/FVC and other indexes can determine whether the airflow is obstructed, so the detection of lung function is an important means of evaluating the treatment effect on COPD (13,14). Studies have found that bronchoalveolar lavage can effectively remove the lung mucus and reduce the lung and airway resistance (15,16). The increase in airway mucus secretion is not normal, especially when the increase in mucus secretion exceeds

Table V. mRNA expression levels of inflammatory factors (mean ± SD).

Groups	IL-6	IL-8	TNF-α	LTB4
One-time lavage				
Before treatment	1.22±0.26	1.56±0.12	0.99±0.13	1.16±0.17
12 days after treatment	0.76±0.21[a,b]	1.22±0.36[a,b]	0.71±0.09[a,b]	0.85±0.23[a,b]
Two-time lavage				
Before treatment	1.26±0.51	1.50±0.53	0.95±0.11	1.08±0.31
12 days after treatment	0.63±0.25[a,c]	1.18±0.25[a,c]	0.58±0.05[a,c]	0.69±0.16[a,c]
Control				
Before treatment	1.25±0.84	1.45±0.32	1.05±0.21	1.25±0.25
12 days after treatment	0.87±0.15[a]	1.32±0.17[a]	0.83±0.03[a]	0.97±0.15[a]

Compared with that before treatment, [a]p<0.01; compared with control group, [b]p<0.05, [c]p<0.01. IL, interleukin; TNF-α, tumor necrosis factor-α; LTB4, leukotriene B4.

Table VI. Expression levels of inflammatory factors (mean ± SD).

Groups	IL-6 (pg/ml)	IL-8 (pg/ml)	TNF-α (mg/ml)	LTB4 (ng/ml)
One-time lavage				
Before treatment	23.67±6.26	28.82±7.65	12.39±2.13	0.42±0.08
12 days after treatment	6.23±3.23[a,b]	8.95±2.53[a,b]	3.51±1.21[a,b]	0.27±0.08[a,b]
Two-time lavage				
Before treatment	23.96±2.51	28.95±6.56	12.11±2.31	0.39±0.12
12 days after treatment	5.12±2.63[a,c]	7.28±3.29[a,c]	2.25±1.65[a,c]	0.21±0.05[a,c]
Control				
Before treatment	24.12±4.84	28.82±5.49	12.45±1.9	0.41±0.09
12 days after treatment	7.97±3.65[a]	10.32±3.55[a]	5.61±1.39[a]	0.33±0.10[a]

Compared with that before treatment, [a]p<0.01; compared with control group, [b]p<0.05, [c]p<0.01. IL, interleukin; TNF-α, tumor necrosis factor-α; LTB4, leukotriene B4.

the normal scavenging capacity of cilia. Thus, the application of bronchoalveolar lavage in removing the airway and lung mucus can significantly increase the cure rate of COPD complicated with pneumonia (17). In this study, the blood gas indexes and biochemical indexes of patients in each group were detected. The results showed that after bronchoalveolar lavage, PaO_2 was significantly increased, but pH and $PaCO_2$ were significantly decreased, and WBC, PCT and CRP were also obviously decreased; with the increase in the number of lavage, the above changes became more obvious. The above results suggested that the patient's lung infection is effectively controlled after bronchoalveolar lavage. Besides, studies have shown that there are a variety of incentives of mucus hypersecretion in COPD, mainly including bacterial infection or activation of mucin gene transcription via inflammatory cells; the production of lung mucus can be effectively lowered by reducing the lung infection (18,19). In this study, the effects of adjuvant therapy with bronchoalveolar lavage on expression of inflammatory factors in lavage fluid of COPD patients complicated with pneumonia were clarified via real-time quantitative PCR and ELISA from the mRNA and protein levels, and the levels of PCT and CRP in peripheral blood were also detected. The results revealed that bronchoalveolar lavage can effectively reduce the inflammatory factors, PCT and CRP levels in lavage fluid, which can effectively control the inflammatory response. Studies have shown that the occurrence of COPD is the extensive inflammatory response caused by the imbalance between inflammatory mediators and anti-inflammatory mediators in the lung, thus resulting in the infiltration of inflammatory cells and inflammatory factors in peripheral airways and alveoli; the effective control of inflammatory factor levels has an important significance in the treatment of COPD complicated with pneumonia (20). It was found in this study that the intermittent application of bronchoalveolar lavage for COPD patients complicated with pneumonia can speed up the patient's prognosis, but the time interval of lavage should be more than 3 days.

In conclusion, the adjuvant therapy with bronchoalveolar lavage can effectively improve the cure rate of COPD patients complicated with pneumonia, effectively remove the excess lung and tracheal mucus, reduce the expression levels of inflammatory factors, and facilitate the control of pulmonary infection and recovery of lung function. However, its specific molecular mechanisms need to be further studied.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonablerequest.

Authors' contributions

HZ and HG collected and analyzed the general patient data. TL performed the adjuvant therapy with bronchoalveolar lavage. JG and GS performed PCR. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Sixth People's Hospital of Nantong (Nantong, China) and informed consents were signed by the patients and/or guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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