Clinical effects of lentinan combined with budesonide inhalation in treating acute exacerbation of chronic obstructive pulmonary disease under mechanical ventilation

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Abstract. In the present study, the clinical efficacy of the immune modulator lentinan combined with inhalation of the corticosteroid budesonide in treating acute exacerbation of chronic obstructive pulmonary disease (AECOPD) under mechanical ventilation was assessed. A total of 72 cases of AECOPD treated at Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China) between June 2016 and September 2017 were enrolled. The AECOPD patients were randomly divided into an experimental group (n=36) and a control group (n=36). All of the patients received ventilator support and endotracheal intubation was performed. The experimental group was orally administered lentinan and budesonide was administered via atomization inhalation through a Y-tube and the control group received only budesonide via Y-tube. After the treatment, airway pressure, the time of mechanical ventilation and the time of stay at the intensive care unit for the experimental group were significantly lower than those for the control group (P<0.001). The plasma levels of adiponectin, D-dimer, interleukin-17 and high-sensitivity C-reactive protein, as well as the pressure of CO_2 in the experimental group were significantly lower than those in control group (P<0.001). Furthermore, the partial O_2 pressure in the experimental group was significantly higher than that in the control group (P<0.001). After the combined treatment, the proportions of $CD3^+$ and $CD4^+$T-cells in the blood were elevated, while the proportion of $CD8^+$T-cells was decreased, compared with those in the experimental group at baseline or the control group post-treatment. In conclusion, the strategy of lentinan treatment combined with budesonide inhalation for AECOPD patients under mechanical ventilation demonstrated improved clinical efficacy compared with budesonide alone. (Chinese Clinical Trial Registry no. ChiCTR1800019088).

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Introduction

COPD is one of the leading causes of death worldwide. Of note, patients with AECOPD and ventilator support in the intensive care unit (ICU) have a higher risk of death and disability (1,2). The duration of mechanical ventilation support has an impact on healthcare costs as well as quality of life (3), and directly affect the prognosis of patients with AECOPD (4,5). It is important to terminate mechanical ventilation early to prevent infection, airway pressure injury and ventilator dependence (6). The standard of management for acute exacerbation inpatients with moderate-to-severe COPD includes supplemental oxygen, non-invasive or invasive mechanical ventilation, short-acting inhaled bronchodilators, systemic corticosteroids and antibiotics (7). Non-pharmacologic management includes ventilator support and administration of supplemental oxygen.

Lentinan is a well-recognized immunomodulator, which is extracted from shiitake (8). AECOPD medications include bronchodilator, anti-infective drugs as required and systemic cortical hormone, but systemic corticosteroid treatment has obvious adverse effects, including hyperglycemia, osteoporosis and vertebral compression fractures. Corticosteroid inhalation therapy is effective and due to its lower dosage, it causes less adverse reactions, and has almost no direct effects on target organs and the blood circulation (9). A previous study has indicated that short-term corticosteroid therapy was as effective as long-term therapy (10). Budesonide suspension (Pulmicort Respules) is currently used as inhaled corticosteroids. Another advantage of atomization inhalation therapy is that corticosteroid may be mixed with bronchodilator, including β2 agonists and anti-cholinergic drugs, which rapidly relieve symptoms. In patients with severe COPD inhaled corticosteroids may reduce the number of exacerbations, the degree of acute exacerbations and the risk of mortality (10).

Studies reporting on the use of corticosteroids and lentinanin critically ill patients with AECOPD on ventilator support are scarce. The present study, the outcome of combined usage of inhaled immunomodulator and corticosteroid in patients with AECOPD was compared. In the present study, the clinical

efficacy of lentinan combined with budesonide inhalation in AECOPD patients receiving ventilation support at the ICU was analyzed, and the serum concentrations of adiponectin (APN), D-dimer (D-D), interleukin (IL)-17 and high-sensitivity C-reactive protein (hs-CRP) were examined. Furthermore, the proportions of $CD3^+$, $CD4^+$ and $CD8^+$T-cells were assessed to evaluate the immunity function of the patients.

Materials and methods

Patients. Written informed consent was obtained from all participants and the present study was approved by the Ethical Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China). The Chinese clinical trial registry number is ChiCTR1800019088. A total of 72 cases of severe AECOPD admitted to the ICU between June 2016 and September 2017 with the requirement of ventilator support were enrolled. All of the patients were divided into two groups by a randomized, double-blinded method, including 36 cases in each group (experimental group: 20 males and 16 females; age, 54.69±5.33 years; and healthy control group: 19 males and 17 females; age, 58.54±8.01). The retrospective patients were all without fatal outcome.

Inclusion and exclusion criteria. All of the patients were diagnosed according to the diagnostic criteria for AECOPD. The inclusion criteria were set in accordance with the Global initiative for chronic Obstructive Lung Disease (GOLD) spirometry definition (11). The exclusion criteria included the diagnosis of pneumonia or pulmonary embolism, asthma, heart failure, thromboembolic disease or restrictive respiratory insufficiency.

Therapies. The experimental group received oral administration of 0.5 g lentinan twice/day (Jinling Pharmaceutical Co., Ltd., Shenzhen, China) and inhalation of 2 ml budesonide (0.5 mg/ml; Astra Zeneca Pharmaceutical Co., Ltd., Shanghai, China) twice a day (bid) over 4 days delivered by a breathing machine Y-tube. The control group only received budesonide atomization inhalation (bid over 4 days) via a breathing machine Y-tube. All patients received the same antibiotic therapy (cephalosporins).

Inclusion criteria included patients with a breathing rate <30/min, a normal cardiovascular function, no abnormal breathing and no airway irritability. The endotracheal intubation time was limited to 120 h.

Clinicopathological characteristics, biochemistry and lung function. The full medical history was reviewed for each patient, and all subjects underwent a full clinical examination and pulmonary function tests. The airway pressure, mechanical ventilation running time, length of ICU stay and rate of trachea incision of patients were recorded. The plasma levels of APN, D-D, serum IL-17 and hs-CRP were measured for all of the patients prior to and after treatment (day 4). A total of 5 ml fasting blood as obtained, and 2 ml plasma was collected in Eppendorf tubes and centrifuged at 1,509 x g for 10 min at 4˚C. The supernatant was put into a new Eppendorf tube and stored in an -80˚C refrigerator. APN (cat. no. 442308) and IL-17 (cat. no. 433918) quantitative ELISA kits were purchased from BioLegend, Inc. (San Diego, CA, USA). The ELISAs for all standards and samples were performed in duplicate. The levels of APN and IL-17 were detected by reading the absorption at 450 nm using an ELISA plate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the protocol of the kits. The concentrations of the analyte proteins were determined through interpolation from a standard curve. ASYSMEX CA-7000 automatic blood coagulation analyzer was used to test the level of D-D through immune turbidimetry (reference value, <0.3 mg/l). A HITACHI 7600 automatic biochemical analyzer (Hitachi, Ltd., Tokyo, Japan) was used to determine the level of hs-CRP (reference value, 0-3 mg/l).

Flow cytometric analysis. The antibodies phycoerythrin-CD3 (cat. no. bs-10498R), fluorescein isothiocyanate-CD4 (cat. no. bs-0647R) and peridinin chlorophyll/cyanine 5.5-CD8 (cat. no. bs-0648R) were purchased from BIOSS (Beijing, China). Whole blood cells were obtained from patients. Cells were fixed with 70% methanol (overnight at 4˚C), permeabilized with 90% ice-cold methanol for 20 min at -20˚C, counted and stained with the aforementioned antibodies at a dilution of 1:1,000 in 10^6/ml cell suspension in dark for 30 min at 4˚C. Following washing, the populations of $CD3^+$, $CD4^+$ and $CD8^+$T-cells were analyzed using a FACSCanto II (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical analysis. Statistical analyses were performed using SPSS software (version 15.0; SPSS, Inc., Chicago, IL, USA). Measurement data were expressed as the mean ± standard deviation. An unpaired t-test was used for comparison between two groups. One-way analysis of variance followed by Tukey's post hoc test was used for determining inter-group differences among multiple groups. Count data were presented as n (%) and analyzed using the χ^2 test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. A total of 80 consecutive patients with severe AECPOD were considered for enrollment. Among them, 3 cases were excluded due to inadequate medical record documentation and 5 patients died during the ICU stay. The baseline characteristics of evaluable cases included in the present analysis are listed in Table I. No differences in age, sex, disease severity, complications and pulmonary function between the experimental and the control group were noted prior to treatment ($P>0.05$).

Airway pressure of breathing machine, mechanical ventilation time and length of ICU stay. A comparison of airway pressure of the breathing machine, mechanical ventilation time and length of ICU stay between the experimental and the control group is provided in Fig. 1. The results indicated that the airway pressure of the breathing machine ($P<0.001$), the mechanical ventilation time ($P<0.001$) and the time of ICU stay ($P<0.001$) in the experimental group were significantly decreased compared with those in the control group.

Serum levels of APN, D-D, IL-17 and hs-CRP. The laboratory analyses indicated that in each group, the plasma levels

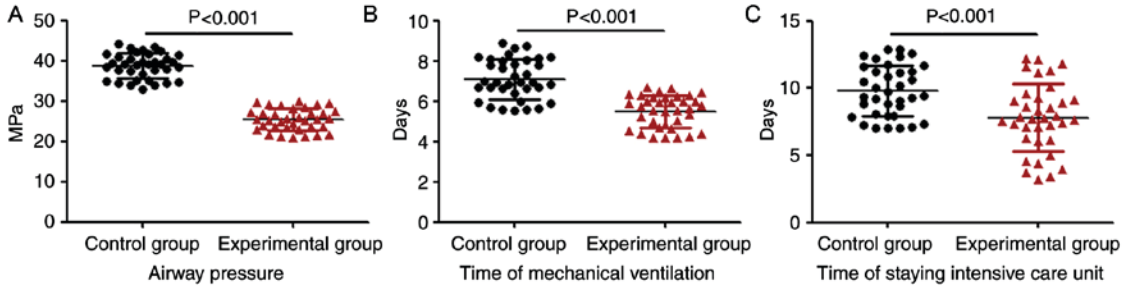

Figure 1. Comparison between patients with acute exacerbation of chronic obstructive pulmonary disease treated by administration of lentinan and budesonide, and those treated with budesonide only, under mechanical ventilation. The (A) airway pressure of the breathing machine, (B) mechanical ventilation time and (C) time of stay at the intensive care unit were compared between the two groups. Values are expressed as the mean ± standard deviation.

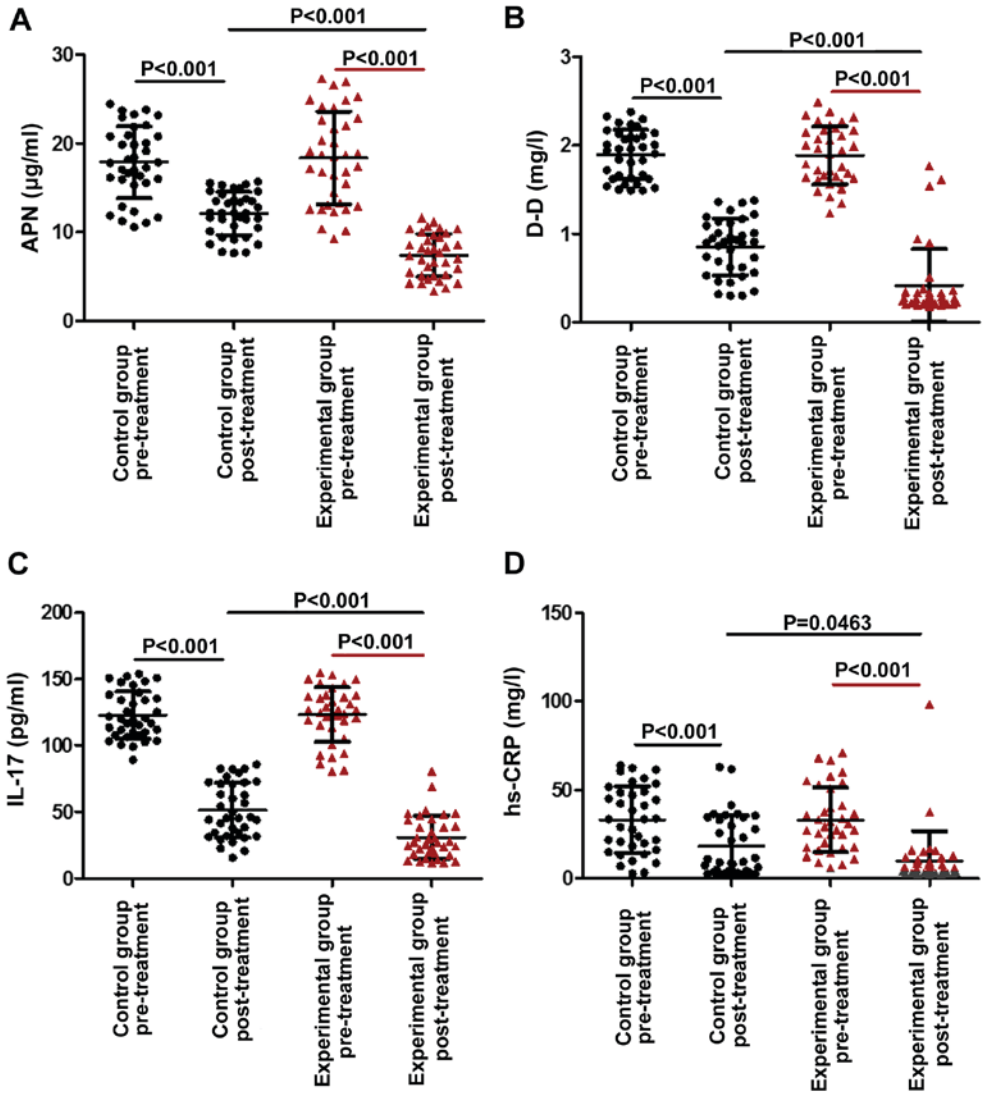

Figure 2. Comparison of the levels of APN, D-D, IL-17 and hs-CRP between AECOPD patients treated with lentinan and budesonide, and those who received budesonide only, under mechanical ventilation. (A) APN, (B) D-D, (C) IL-17 and (D) hs-CRP levels in the two groups prior to and post-treatment are provided. Values are expressed as the mean ± standard deviation. AECOPD, acute exacerbation of chronic obstructive pulmonary disease. D-D, D-dimer; IL, interleukin; APN, adiponectin; hs-CRP, high-sensitivity C-reactive protein.

of APN, D-D, IL-17 and hs-CRP after treatment were obviously lower than those at baseline (P<0.05). Furthermore, the levels of APN (P<0.001), D-D (P<0.001) and IL-17 (P<0.001) in the experimental group were significantly lower than those in the control group, suggesting that administration of lentinan in addition to budesonide in the experimental group further reduced the level of APN, D-D and IL-17 (Fig. 2A-C). Regarding the serum levels of hs-CRPs, the difference between the experimental and the control group after the treatment was not significant (Fig. 2D). After treatment, as

Table I. Baseline characteristics of the patients.

Parameter	Control (n=36)	Experimental group (n=36)
Age (years)	54.69±5.33	58.54±8.01
Sex (male/female)	19 (26.39%)/17 (23.61%)	20 (27.78%)/16 (22.22%)
BMI (kg/m^2)	30.12±6.87	31.81±7.66
FEV_1%	42.88±15.02	41.57±16.45
FEV_1/FVC	40.87±17.98	41.71±17.09
GOLD stage		
1	16 (22.22%)	12 (16.67%)
2	11 (15.28%)	10 (13.89%)
3	6 (8.33%)	7 (9.72%)
4	5 (6.94%)	5 (6.94%)

Values are expressed as the mean ± standard deviation or as n (%). BMI, body mass index; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; GOLD, Global initiative for chronic Obstructive Lung Disease.

Table II. Comparison of blood gas pressure parameters between the two groups.

	Experimental group (n=36)		Control (n=36)	
Parameter	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
PaO_2 (mmHg)	50.58±7.35	78.82±8.57[a,b]	51.39±5.76	67.39±11.25[a]
PCO_2 (mmHg)	69.91±9.46	46.71±9.28[a,c]	68.39±10.42	53.15±9.72[a]

Values are expressed as the mean ± standard deviation. [a]P<0.001 compared with pre-treatment; [b]P<0.001 compared with control group post-treatment; [c]P<0.05 compared with control group post-treatment. PaO_2, partial oxygen pressure.

presented in Table II, the partial oxygen pressure (PaO_2) in two groups was significantly increased compared with the baseline value (P<0.001), while the CO_2 pressure (PCO_2) was significantly decreased (P<0.001). Furthermore, the PaO_2 in the experimental group was significantly higher than that in the control group (P<0.001), while the PCO_2 was significantly lower (P<0.05).

Proportion of CD3$^+$, CD4$^+$ and CD8$^+$ T-cells. The effect of lentinan combined with budesonide on the proportions of CD3$^+$, CD4$^+$ and CD8$^+$ T-cells was then examined. As presented in Fig. 3, the percentages of CD3$^+$ and CD4$^+$ T-cells were significantly increased after treatment, compared with those at baseline and in the control group (P<0.01). In addition, the percentage of CD8$^+$ T-cells in the experimental group was decreased compared with that at baseline and in the control group (P<0.001).

Discussion

COPD has become an important public health problem due to the high mortality, as well as the social and economic burden associated with it. At present, COPD is the fourth most common cause of death worldwide. The World Bank and the World Health Organization have announced that COPD will be the world's fifth economic burden in 2020 (12). AECOPD in combination with respiratory failure is the most important reason for readmission of COPD patients to the hospital. Thus, it is of great significance to improve the means of prevention of AECOPD, and in particular, to improve the success rate of treating AECOPD combined with respiratory failure. Significant progress has been made regarding the clinical application of mechanical ventilation in the treatment of AECOPD, which has greatly improved the quality of life of affected patients (13).

COPD is a type of chronic bronchitis with characteristics of airflow obstruction and/or emphysema and progressive development, which is associated with airway hyper-responsiveness. The long-term use of a breathing machine leads to an increased incidence of ventilator-associated pneumonia (VAP) (14). For AECOPD patients with a VAP resistance phenomenon, generic drug-resistant *boydii* Acinetobacter strains, fungal infection and drug resistance have been observed (15), and adverse effects including an increase of airway damage were also observed (16). However, atomization therapy with budesonide may reduce airway resistance, breathing machine plenum pressure drop and airway pressure damage (17). The effect of standard doses of lentinan combined with budesonide for AECOPD has been rarely reported.

APN, secreted by fat cells, is an endogenous anti-inflammatory factor in airway epithelial cells, is released by autocrine and paracrine systems, and is a novel marker of airway

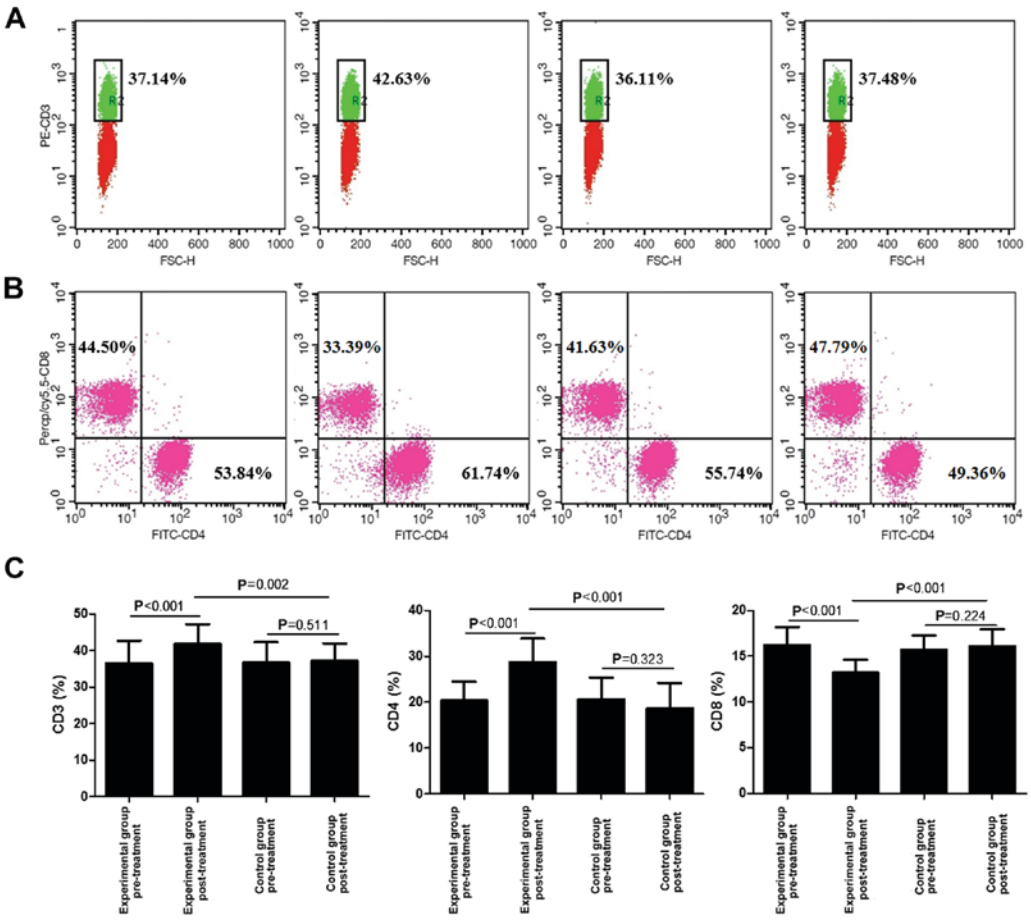

Figure 3. Analysis of $CD3^+$, $CD4^+$ and $CD8^+$ T cells in the blood of acute exacerbation of chronic obstructive pulmonary disease patients treated with lentinan and budesonide, and those who received budesonide only, under mechanical ventilation. (A) FACS plots of $CD3^+$ T cells; (B) FACS plots of $CD4^+$ and $CD8^+$ T cells; (C) Average percentages of $CD3^+$, $CD4^+$ and $CD8^+$ T cells, calculated from the FACS analysis, prior to and post-treatment are provided. Values are expressed as the mean ± standard deviation. FACS, fluorescence-assisted cell sorting; FITC, fluorescein isothiocyanate; Percp, peridinin chlorophyll; cy, cyanine; FSC, forward scatter; PE, phycoerythrin.

inflammation with a high predictive value for AECOPD. It was reported that the severity of COPD and the expression levels of APN were positively correlated (18,19). COPD patients with chronic hypoxia, infection and accumulated inflammatory cytokines may develop endothelial damage, which eventually promotes thrombosis (20). D-D reflects the high coagulation state in the body and secondary fibrinolytic hyperfunction. IL-17 promotes the release of other associated inflammatory cytokines, causing neutrophil aggregation in the lung and damage to the structure of the airways (21). AECOPD is mostly caused by infection, with bacterial infection accounting for 40-50% of AECOPD incidence (22). To the best of our knowledge, hs-CRP is a sensitive indicator that reflects bacterial infections.

The present study indicated that the airway pressure, usage time of breathing machine, length of stay at the ICU in the experimental group was significantly lower than that in the control group. The levels of APN, D-D, IL-17 and hs-CRP of AECOPD patients at baseline were higher than those after treatment, indicating the presence of a certain extent of infection, a high coagulation state and fibrinolytic hyperfunction in AECOPD patients. The levels of APN, D-D, IL-17 and hs-CRP after treatment were significantly lower than those prior to treatment in each group, suggesting that the infection, high coagulation state and fibrinolytic state were relieved. The levels of APN, D-D, IL-17 and hs-CRP in the experimental group were significantly lower than those in the control group, suggesting that the combined use of lentinan with budesonide is better than monotreatment with budesonide, which may therefore be worthy of implementation in the clinic. In comparison with those in the control group, the PaO_2 was significantly increased and the PCO_2 was significantly increased, suggesting that combination treatment of lentinan with budesonide provided a better outcome. Flow cytometric analysis suggested that combination of lentinan with budesonide greatly improved the immunity function of patients with AECOPD, which may contribute to the improved clinical outcome.

In the present study, only single dose of lentinan was administered to the experimental group, which indicated that lentinan could improve the immunity function and clinical outcome of patients with AECOPD. However, the optimal dose of lentinan were not determined in the current study. This represents a limitation and more studies are required to explore the clinical outcome of multiple dosages of lentinan and to analyze the optimal dose in AECOPD patients.

In summary, a significant benefit for AECOPD patients receiving mechanical ventilation was observed by combination treatment with lentinan and budesonide, with a shorter

duration of mechanical ventilation, improvement of the serum levels of APN, D-D, IL-17 and hs-CRP, as well as the PaO_2 and PCO_2, as compared with the outcomes of treatment with budesonide alone. The present study provided a novel strategy for the combined administration of these medications.

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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 reasonable request.

Authors' contributions

GZ conceived and designed the study, wrote the manuscript and was involved in its critical revision. GZ and JS retrieved the samples and performed the experiments, they collected, processed and analysed the data and interpreted the results.

Ethical approval and consent to participate

Written informed consent was obtained from all participants and the present study was approved by the Ethical Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests regarding this study.

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