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Original Article

Effects of adjuvant pidotimod therapy on levels of inflammatory factors and expressions of serum GM-CSF and KL-6 in elderly patients with mycoplasma pneumonia

Lin Xu[1,3], Ying Zhao[1], Shaokui Wu[5], Qingying Song[4], Zhanghong Ouyang[2], Xiangyan Zhang[1,3], Cheng Zhang[1,3]

Departments of [1]Respiratory and Critical Care Medicine, [2]Development Planning Office, Guizhou Provincial People's Hospital, Guiyang 550002, Guizhou Province, China; [3]Medical School of Guizhou University, Guiyang 550025, Guizhou Province, China; [4]Department of Anesthesiology, The Second Affiliated Hospital of Guizhou College of Traditional Chinese Medicine, Guiyang 550003, Guizhou Province, China; [5]School of Physical Education, Guizhou University 550025, Guiyang, Guizhou Province, China

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Abstract: Objective: To observe the intervention effects of adjuvant pidotimod therapy on the serum inflammatory factor and GM-CSF and KL-6 expression levels in elderly mycoplasma pneumonia patients. Methods: Elderly patients (n=104) diagnosed with mycoplasma pneumonia were divided into a control group (52 cases, given conventional anti-infective therapy combined with ambroxol) and a research group (52 cases, given conventional anti-infective therapy combined with ambroxol + pidotimod) according to the different treatment methods each patient was administered. The pulmonary function indexes (FVC, FEV1, FEV1/FVC), the serum inflammatory factor levels (interleukin (IL)-6, IL-8, the tumor necrosis factor α), the serum granulocyte-macrophage colony-stimulating factor (GM-CSF), and the Krebs von den Lungen-6 (KL-6) expression levels were measured before and after the treatment. The cough stopping times, the rale disappearance times, the hospital stay durations, the overall response rates, the incidences of adverse reactions during the administration, and the recurrence rates at 3, 6, and 12 months after the treatment were recorded. Results: The research group had shorter cough stopping times, rale disappearance times, and hospital stays than the control group (all P<0.05). After the treatment, the FVC, FVE1, and FVE1/FVC levels in both groups were increased, and the research group had higher levels than the control group (all P<0.05). After the treatment, the serum tumor necrosis factor α, IL-6, IL-8, GM-CSF, and KL-6 levels in the two groups were significantly decreased, and the levels of these indicators in the research group were significantly lower than they were in the control group (all P<0.05). The total overall treatment response rate was higher, and the recurrence rate at 12 months after the treatment was significantly lower in the research group than they were in the control group (P<0.05). Conclusion: Adjuvant pidotimod therapy in the treatment of elderly patients with mycoplasma pneumonia can ameliorate patients' inflammatory responses and pulmonary functions, and reduce the serum GM-CSF and KL-6 factor levels, as well as the recurrence rate. Moreover, the combined medication is safe, and no significant increase in toxicity was found.

Keywords: Pidotimod, ambroxol, elderly mycoplasma pneumonia patients, serum inflammatory factor, pulmonary function, safety

Introduction

Mycoplasma pneumonia, accounting for 10% of all pneumonia types, is caused by *Mycoplasma pneumoniae* infection leads to local tissue damage and pulmonary function decline in patients and carries the risk of death in severe cases [1]. Children and the elderly are regarded as the most susceptible groups, due to their poor immune function [2]. At present, antibiotics are a widely used treatment for mycoplasma pneumonia. However, the improvements in cough and sputum symptoms and the recovery of pulmonary function in patients treated with single antibiotics are unsatisfying [3]. Therefore, other drugs are often required. Ambroxol, a

mucolytic drug, helps make phlegm easier to cough up [4]. Zhu found that the overall response rate of ambroxol oral liquid in the treatment of infant bronchial pneumonia was as high as 90%, far exceeding that of ambroxol hydrochloride granules [5]. Pidotimod, a commonly used clinical immunomodulator, can significantly improve the immune function and enhance the therapeutic effect in people with weak immunity, and it is used as an auxiliary drug in anti-infective treatment [6]. Feng et al. pointed out that the therapeutic efficacy of adjuvant pidotimod treatment for children with mycoplasma pneumonia was as high as 90.9%, which was significantly higher than the efficacy in the control group (62.5%), and it can adjust the balance of Th17/CD4+ CD25+ Treg to achieve the treatment goal [7]. The above two drugs have a good clinical effectiveness in the treatment of bronchial pneumonia in children, but there are few studies on the effects of the combined application in the treatment of elderly patients with mycoplasma pneumonia. Therefore, this retrospective study aims to explore the effects of adjuvant pidotimod therapy on the serum expression levels of the inflammatory factors, the granulocyte-macrophage colony-stimulating factor (GM-CSF), and Krebs von den Lungen-6 (KL-6) expression levels in elderly mycoplasma pneumonia patients.

Materials and methods

General data

From May 2017 to April 2020, 104 patients with mycoplasma pneumonia were selected for this retrospective study. They were divided into a control group (52 cases, given conventional anti-infective therapy combined with ambroxol) and a research group (52 cases, given conventional anti-infective therapy combined with ambroxol + pidotimod) according to the different treatment methods each patient was administered. This study was approved by the ethics committee of our hospital after a review of the safety of the medication (approval No. GZPPH-001) and after the patients signed the informed consent forms.

Inclusion criteria: (1) Patients who were diagnosed with mycoplasma infection using complement fixation tests and indirect hemagglutination assays [8]. (2) Patients over 65 years old. (3) Patients who were infected for the first time. (4) Patients with normal immune functions. (5) Patients who were not administered anti-infective drugs or immunomodulatory drugs in the week before their admission. (6) Patients who signed the written informed consent forms.

Exclusion criteria: (1) Patients with malignant tumors, high blood pressure, diabetes, etc. (2) Patients with a history of drug allergies. (3) Patients with cognitive dysfunctions. (4) Patients with other infections and trauma.

Methods

The patients in the two groups were administered conventional treatment and azithromycin (H20051818, Qingdao Jinfeng Pharmaceutical Co., Ltd.) mixed with 250 mL of normal saline intravenously. In addition to this treatment, the patients in the control group were administered oral ambroxol (H20040317, Beijing Hanmei Pharmaceutical Co., Ltd., China) 20 mL/time, twice a day for 7 days. The research group was treated with oral pidotimod in addition to the ambroxol (H20010091 Sun Pharmaceutical Co., Ltd., China) 0.8 g/time, twice a day for 7 days.

Before and after the treatment, 4 mL of fasting venous blood was collected from each patient. The serum was separated and stored in a low temperature environment (-80°C), and the serum inflammatory factor levels (interleukin (IL)-6, IL-8, tumor necrosis factor α (TNF-α)), GM-CSF, and KL-6 were measured [9]. The kits were stored at 2~8°C, re-dissolved and mixed with standard product to prevent the substrate from deoxidizing. Each pipette tip was replaced after the sample was added. The test tube was shaken to fully mix the samples, which were reacted for 15 min at room temperature (25~28°C). The standard product was diluted in multiples and then added to the detection hole. Every hole was injected with 300 uL of washing solution. The interval between the injection and the suction was 15~30 s. After completion, the samples were taken out and placed in aluminum foil and sealed at 4°C. The standard products with different concentrations were added into the wells. Then the reaction wells were sealed and incubated at 25~28°C for 2 h. All the serum samples were mixed with the same number of samples after 50 μL of sample analysis solution was added,

then they were washed 5 times and patted dry with absorbent paper. The plate was sealed with 100 μL of biotinylated antibody working solution and incubated at 25~28°C for 1 h. Then the enzyme conjugate working solution (100 μL) was added. The reaction well was sealed and incubated at 25~28°C for 20 min, added with 100 ul color reagent, incubated for 20 min. The OD value was determined after 50 μL of stop solution was added.

Outcome measures

Before and after the treatment, a pulmonary function detection system (Shanghai Hanfei Medical Equipment Co., Ltd., China) was used to measure and calculate each patient's forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and FEV1/FVC [10].

The IL-6, IL-8, TNF-α, GM-CSF, and KL-6 levels were measured and compared before and after the treatment. Enzyme-linked immunosorbent assays were used to determine the expressions of the above factors. TNF-α, IL-6, IL-8 and GM-CSF, KL-6 ELISA kits were purchased from Wuhan Boster Bioengineering Co., Ltd., China and the procedures were carried out in strict accordance with the kits' instructions.

The cough stopping times, the rale disappearance times, and hospital stay durations were recorded.

After the treatment, when patient's symptoms such as fever, rales, and cough almost completely disappeared, this was classified as markedly effective. When the above symptoms were notably improved after the treatment, this was classified as effective. No significant improvement after the treatment from such symptoms was classified as ineffective [11]. Total overall response rate = (total number of cases-number of ineffective cases)/total number of cases *100%.

The occurrence of adverse reactions such as gastrointestinal symptoms, skin damage, and nervous system damage in the patients after the treatment administration were recorded. The rate of adverse reactions = (number of gastrointestinal symptoms, skin damage, and nervous system damage cases)/total number of cases *100%. For the patients who had two types of adverse reactions, the more serious symptom was recorded. If the above-mentioned adverse reactions occurred, the patients were treated with symptomatic measures such as protecting the gastric mucosa, and reducing the inflammation of the skin and the nervous system damage according to the patient's condition. If the patient's symptoms were severe, the above-mentioned drug treatment was stopped.

The recurrence at 3, 6, and 12 months after the treatment were recorded within the 1-year follow-up period. Complement binding assays and indirect hemagglutination tests were used to determine the IgG and IgM antibody expressions. A positive test result for IgG or IgM in a sputum culture or a positive test result for *Mycoplasma pneumoniae* were considered to be a recurrence. The recurrence rate = number of recurrences/total number of cases *100%.

Statistical analysis

SPSS 22.0 software was used for the statistical analyses. GraphPad Prism 7.0 was used to create the graphs. The measurement data were expressed as the mean ± standard deviation ($\bar{x}$±sd). The comparisons of the data before and after the treatment within a group were tested using paired-sample t tests. Independent t tests were carried out for the comparisons between groups before and after the treatment. The count data were expressed as a number/percentage (n/%), and chi-square tests were used for the rate comparisons between the two groups. $P<0.05$ indicated that the results were significantly different.

Results

Comparison of the baseline data

No statistically significant differences were found in terms of age, gender, course of the disease, or body mass index between the two groups (all $P>0.05$). See **Table 1**.

Comparison of the lung function indexes before and after the treatment

There were no significant differences in the FVC, FVE1, or FVE1/FVC values before the treatment in both groups (all $P>0.05$). After the treatment, the FVC, FVE1, and FVE1/FVC values in the two groups were increased, and the

Table 1. Comparison of the baseline data ($\bar{x}$±sd)

Item	Control group (n=52)	Research group (n=52)	t	P
Age (year)	72.9±4.8	73.1±4.9	0.667	0.507
Gender (male/female, case)	35/17	37/15	0.345	0.731
Course of disease (day)	3.2±0.5	3.3±0.5	1.020	0.310
Body mass index (kg/m^2)	24.21±2.85	24.34±2.91	0.230	0.818

Table 2. Comparison of the lung function indexes before and after the treatment ($\bar{x}$±sd)

Pulmonary function	Control group (n=52)	Research group (n=52)	t	P
FVC (L)				
Before treatment	2.03±0.24	2.07±0.26	0.667	0.507
After treatment	2.42±0.31*	2.67±0.35*	3.781	0.001
FEV1 (L)				
Before treatment	1.01±0.14	1.02±0.15	0.345	0.731
After treatment	1.32±0.22*	1.56±0.26*	4.983	0.001
FEV1/FVC (%)				
Before treatment	49.75±3.67	49.28±3.71	0.637	0.526
After treatment	54.55±3.08*	58.43±3.34*	6.039	0.001

Note: Compared with before the treatment, *P<0.05. FVC: forced vital capacity; FEV1: forced expiratory volume in 1 second.

Table 3. Comparison of the serum inflammatory factor levels before and after the treatment ($\bar{x}$±sd, pg/mL)

Serum inflammatory factors	Control group (n=52)	Research group (n=52)	t	P
IL-6				
Before treatment	46.94±4.91	47.05±4.93	0.112	0.911
After treatment	21.63±2.33*	15.31±1.37*	16.534	0.001
IL-8				
Before treatment	8.61±1.38	8.73±1.35	0.440	0.660
After treatment	6.22±1.02*	4.31±0.63*	11.265	0.001
TNF-α				
Before treatment	89.64±8.55	90.33±8.67	0.401	0.690
After treatment	53.62±5.87*	46.55±4.71*	6.643	0.001

Note: Compared with before the treatment, *P<0.05. IL: interleukin; TNF-α: tumor necrosis factor-α.

research group had higher levels than the control group (all P<0.05). See **Table 2**.

Comparison of the serum inflammatory factor levels before and after the treatment

There were no significant differences in the serum inflammatory factor levels between the two groups before the treatment (all P>0.05). After the treatment, the serum TNF-α, IL-6, and IL-8 levels in both groups were significantly decreased, and the levels of these indicators in the research group were significantly lower than the levels in the control group (all P<0.05). See **Table 3**.

Comparison of the serum GM-CSF, KL-6 levels before and after the treatment

No statistically significant differences were identified in the GM-CSF or KL-6 levels between the two groups before the treatment (both P>0.05). After the treatment, the GM-CSF and KL-6 levels in both groups were significantly decreased, and the levels of these indicators in the research group were significantly lower

Table 4. Comparison of the serum GM-CSF and KL-6 levels before and after the treatment ($\bar{x}$±sd)

Group	Case (n)	GM-CSF (pg/mL)		KL-6 (U/mL)	
		Before treatment	After treatment	Before treatment	After treatment
Control group	52	43.11±4.26	35.33±3.87*	572.31±54.21	361.22±37.71*
Research group	52	43.51±4.52	30.16±3.09*	573.99±50.36	300.19±30.87*
t		0.455	7.382	0.161	8.855
P		0.650	0.001	0.873	0.001

Note: Compared with before the treatment, *P<0.05. GM-CSF: granulocyte-macrophage colony-stimulating factor. KL-6: Krebs von den Lungen-6.

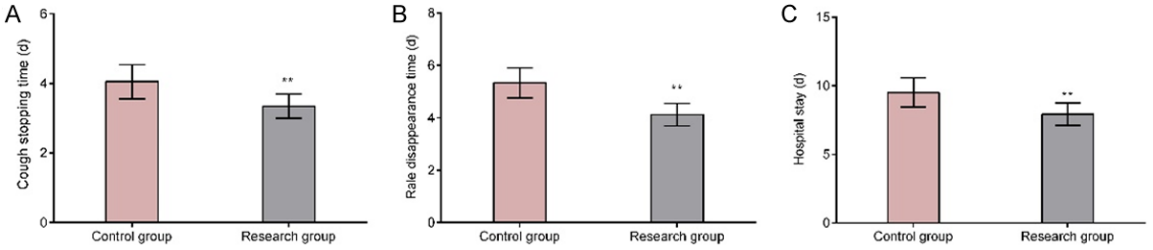

Figure 1. Comparison of the cough stopping times, the rale disappearance times, and the hospital stay durations. A: Cough stopping times. B: Rale disappearance times. C: Hospital stay durations. **P<0.01.

Table 5. Comparison of the overall response rates (n, %)

Group	Case (n)	Ineffective	Effective	Markedly effective	Overall response rate
Control group	52	10 (19.23)	18 (34.62)	24 (46.15)	42 (80.77)
Research group	52	3 (5.77)	19 (36.54)	30 (57.69)	49 (94.23)
χ^2		4.308	0.042	1.387	4.308
P		0.038	0.838	0.239	0.038

Table 6. Comparison of the incidences of adverse reactions (n, %)

Group	Case (n)	Gastrointestinal symptoms	Skin damage	Nervous system damage	Incidence
Control group	52	3 (5.77)	1 (1.92)	1 (1.92)	5 (9.62)
Research group	52	3 (5.77)	2 (3.85)	2 (3.85)	7 (13.46)
χ^2		0.001	0.343	0.343	0.377
P		1.001	0.558	0.558	0.539

than the levels in the control group (both P<0.05). See **Table 4**.

Comparison of the cough stopping times, the rale disappearance times, and the hospital stay durations

The research group had shorter cough stopping times, rale disappearance times, and hospital stays than the control group (P<0.01). See **Figure 1**.

Comparison of the overall response rates

The total overall treatment response rate in the research group was higher than the overall treatment response rate in the control group (P<0.05). See **Table 5**.

Comparison of the incidences of adverse reactions

There was no significant difference in the incidences of adverse reactions between the groups (P>0.05). See **Table 6**.

Comparison of the 3, 6, and 12 month recurrence rates after the treatment

The recurrence rates in the control group at 3, 6 and 12 months were 3.85% (2/52), 7.69%

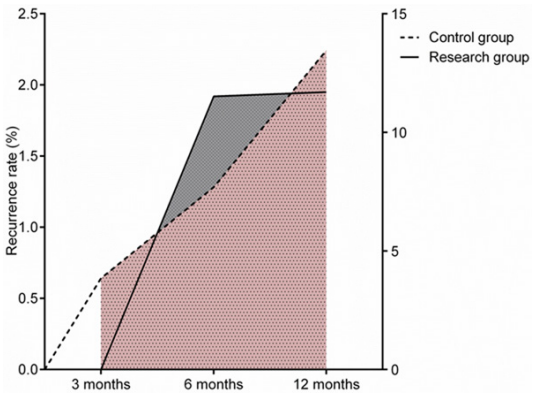

Figure 2. Comparison of the recurrence rates at 3, 6, and 12 months after the treatment.

(4/52), and 13.46% (7/52), respectively. The recurrence rates at 3, 6, and 12 months in the research group were 0 (0/52), 1.92% (1/52), and 1.92% (1/52), respectively. There were no significant differences in the recurrence rates at 3 and 6 months after the treatment (both $P>0.05$). Compared with the control group, the research group had a significantly lower recurrence rate at 12 months after the treatment ($P<0.05$). See **Figure 2**.

Discussion

At present, drug therapy is the main clinical treatment for elderly mycoplasma pneumonia patients [12]. Ambroxol is widely used to treat pneumonia. It can improve the viscosity of the sputum by reducing the fibrous activity of acidic protein in the sputum and reduce the secretions of the airway glands to promote sputum dilution [12-14]. As an immunomodulator, pidotimod can act on the body and achieve auxiliary antibacterial and antiviral effects by improving the patient's own immunity [15-18].

The research group had a superior clinical effectiveness, and the incidence of adverse reactions was not significantly different when compared with the control group. This indicated that the combined treatment can enhance the clinical effectiveness safely and without significant toxicity. Yin et al. observed pidotimod combined with antibiotics in the treatment of mycoplasma pneumonia and found that the patients in the research group had better outcomes than the control group in terms of their serum inflammatory factor levels, immune indicators, and total effective rates, which is consistent with the results of this study [19]. At the same time, in our study, we found that that the FVC, FEV1, and FVE1/FVC values in both groups were increased after the treatment, and the lung function indicator levels in the research group were higher than the lung function indicator levels in the control group. In addition, the cough stopping times, the rale disappearance times, and the hospital stays in the research group were shorter than they were in the control group, suggesting that adjuvant pidotimod therapy in treating elderly mycoplasma pneumonia patients can significantly and more quickly improve their lung functions and recoveries, which can effectively shorten their hospital stays.

TNF-α, IL-6, and IL-8 are common inflammatory factors. During the occurrence and development of pneumonia, the continuous aggravation of the inflammatory response is one of the important factors leading to the continuous decline of the patient's lung function [20, 21]. The up-regulation of the inflammatory factors indicates that the lung pathogens proliferate in large quantities and the patient's condition deteriorates [22-24]. Low levels of TNF-α can protect antibodies and enhance autoimmunity, but in patients with mycoplasma pneumonia, its concentration is significantly increased, and it becomes an inflammatory mediator by itself, which induces gradual inflammation. IL-6 can promote the production of antibodies, meanwhile inducing the differentiation of T lymphocytes and promoting the release of large amounts of inflammatory factors. IL-8 can activate neutrophils, promote the release of lysosomal enzymes and superoxide, and promote the aggravation of inflammation. In this study, the research group had better treatment outcomes in their serum inflammatory factor levels, showing that ambroxol combined with pidotimod can significantly improve patients' pulmonary inflammation and restore their lung function, so it is more effective than ambroxol treatment alone. We analyzed the experimental data and posit that pidotimod combined with ambroxol can effectively improve the viscosity of respiratory tract sputum, promote sputum, and restore respiratory function. Pidotimod can promote specific and non-specific immune responses, enhance the activity of macro-

phages and neutrophils, activate natural killer cells, accelerate the proliferation of lymphocytes, stimulate interleukin-a and r-interferon, and promote the cellular immune response, thereby reducing the TNF-α, IL-6, and IL-8 levels and reducing inflammation. Hence, the immune indicators, the total overall response rate, and the lung function in the research group were better than they were in the control group.

GM-CSF, the structure of the hematopoietic growth factor, is produced by damaged endothelial cells after being stimulated, to promote the production, proliferation and differentiation of granulocytes and monocytes [25]. In patients with pneumonia, the cellular immunity is impaired and the active state of lymphocytes is inhibited, then the expression level of GM-CSF is increased with the severity of the patient's condition [25]. KL-6 is mainly derived from alveolar type II epithelial cells, which are closely related to interstitial lesions in patients with pneumonia [26]. During the progression of pneumonia, the structure and function of alveolar type II epithelial cells change, the KL-6 level is significantly increased. The destruction and regeneration of the patient's alveolar epithelial cells can be determined via KL-6, and its concentration is directly proportional to the state of pneumonia [26]. In this study, the research group had lower levels of GM-CSF and KL-6 than the control group, which suggests that the combined administration can inhibit the local inflammation, improve the patient's own immunity, and effectively protect the patient's alveolar epithelial and endothelial cells, thereby down-regulating the GM-CSF and KL-6 expression levels and achieving a good therapeutic effect eventually. Wang et al. pointed out that the combined application of pidotimod with loratadine can effectively improve the clinical efficacy and quality of life of patients with allergic rhinitis, regulate the imbalance of Th1/Th2 cytokines in patients, and reduce the serum levels and the inflammatory factor levels, and EOS and GM-CSF, which are well tolerated by patients [27]. The mentioned findings are similar to the results of our study. Secondary recurrence is a common situation in elderly patients with mycoplasma pneumonia, which increases the risk of drug resistance and is an important factor affecting patient prognosis. In this study, the research group had a lower recurrence rate than the control group, showing that the long-term effect of the combined administration on patients was better than that of ambroxol alone, but its mechanism is still unclear.

This is a single-center study with a small sample size and a short follow-up time, so the experimental outcome may be biased. Thus, the experimental design is expected to be improved in the future.

In summary, in the treatment of elderly mycoplasma pneumonia, adjuvant pidotimod therapy can relieve symptoms faster than ambroxol alone, and it inhibits inflammation, improves pulmonary function, down-regulates patients' serum GM-CSF and KL-6 levels, and improves the clinical effectiveness. Also, the combined administration is very safe and without any significant toxicity.

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Disclosure of conflict of interest

None.

Address correspondence to: Cheng Zhang and Xiangyan Zhang, Department of Respiratory and Critical Care Medicine, Guizhou Provincial People's Hospital, No. 52 East Zhongshan Road, Guiyang 550002, Guizhou Province, China. Tel: +86-0851-85273966; Fax: +86-0851-85273966; E-mail: Chengzhang66@protonmail.com (CZ); E-mail: zxy35762@126.com (XYZ)

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