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Clinical effect of treating secondary asthma attacks of children *Mycoplasma pneumoniae* with combined therapy of montelukast and azithromycin

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Abstract. – OBJECTIVE: To discuss the clinical effects of treating secondary asthma attacks of children *Mycoplasma pneumoniae* with combined therapy of montelukast and azithromycin.

PATIENTS AND METHODS: 96 children patients diagnosed with secondary asthma attacks of Mycoplasma pneumonia were enrolled in this study. They were randomly divided into two groups: the control group (n=49) and the observation group (n=47). Patients in the control group received combined therapy using azithromycin and bronchodilators or glucocorticoid, and patients in the observation group received a combined therapy of montelukast, azithromycin and bronchodilators or glucocorticoid. The lung function indexes, T lymphocyte subpopulation, cytokines levels, positive rate of IgG and IgM, asthma control rate and recurrence rate were compared between groups before and after treatment.

RESULTS: The levels of V-T, t-PTEF/t-E, MTIF/MTEF and TEF25/PTEF in both groups increased after treatment, but we observed a more significant improvement in the observation group. The CD4+ and CD4+/CD8+ levels in both groups also increased after the intervention, while the level of CD8+ decreased. The IL-10, IL-17 and TGF-β levels decreased more intensely in the observation group.

DISCUSSION: The positive rate of IgG and IgM in both groups decreased significantly after the intervention. In the observation group, the asthma control rate was higher while the recurrence rate was lower. Although montelukast had little effect on improving the immune function, it was certainly beneficial for controlling the symptoms of asthma and improving the prognosis.

CONCLUSIONS: Using combined therapy of montelukast and azithromycin for treating the secondary asthma attacks of children mycoplasma pneumonia can relieve immunological and inflammatory reactions and improve the lung function.

Key Words:
Montelukast, Azithromycin, Mycoplasma pneumonia, Children asthma, Lung function, T lymphocyte, Cytokines.

Introduction

Mycoplasma pneumoniae is one of the most common pathogens for respiratory tract infection. Mycoplasma is a bacterial parasite without cell wall that can spread through spittle. It can occur at any age, but children before the school age are more vulnerable[1]. One of the reasons for this may be related to the fact that immunologic function in children is not fully developed. The clinical manifestation of *Mycoplasma pneumoniae* infection is not typical and may be easily misdiagnosed. During the early stages of the infection, sick children may show symptoms such as coughing and fever. As the condition deteriorates, children may develop cough-type-asthma[2]. The cough-type-asthma is featured by the main symptom of coughing, accompanied by continuous airway hyperreactivity and inflammatory response. Delayed treatment will lead to persistent adult asthma[3]. Usually, the outcome of treatment by single antibiotic is not ideal, and the wide application of antibiotics can lead to an increase in drug-resistant *Mycoplasma pneumoniae* strains.

When the secondary asthma attack of children mycoplasma pneumonia occurs, the level of inflammatory factors usually increases, and we also see a surge in eosinophilic granulocytes. The eosinophilic granulocyte will release type 1 cysteinyl leukotriene receptors (CYSLTR1), which can be distributed inside the airway and cause a series of airway reactions[4]. The montelukast has high selectivity for CYSLTR1s, thus can effectively control them and limit the accumulation of inflammatory factors, decrease the secretion of grume, block the bronchial constriction, relax bronchial smooth muscles and improve the lung function[5,6]. In this study, the secondary asthma attacks of children mycoplasma pneumonia were treated with combined therapy of montelukast

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and azithromycin and promising results were achieved.

Patients and Methods

Patients

From November 2014 to December 2015, 96 children diagnosed with secondary asthma attacks caused by children *Mycoplasma pneumoniae* were enrolled in this study. Inclusion criteria: (i) Patients conformed to mycoplasma pneumonia diagnosis criteria formulated by Chinese Medical Association; (ii) Patients were 2-14 years old; (iii) Their condition was combined with an asthma attack.

Exclusion criteria: (i) Patients with simple asthma attack; (ii) Patients with abnormal development of airway, congenital diseases, inherited metabolic and autoimmune diseases; (iii) Patients allergic to azithromycin and montelukast; (iv) Those unable to complete the study, and those with incomplete follow-up data.

This study obtained the approval of Ethic Committee in our hospital and patients' families signed informed consent forms. Patients were randomly divided into two groups: the control group (n=49) and the observation group (n=47). There were 23 males and 26 females in the control group; aged between 3 to 14 years (average=6.8±2.6 years). The course of disease was 5 to 28 days (average=8.7±3.2 days). In the observation group, we had 26 males and 21 females; aged 2 to 14 years (average=6.7±3.2 years). The course of disease was 4 to 25 days (average=8.8±4.2 days). General patients' data in two groups were comparable.

Methods

Patients in both groups received regular anti-infection therapy, while antibiogram tests were conducted on the sputum and blood cultures. All patients received treatment for fever, cough, eliminating phlegm and oxygen uptake. Patients in the control group were treated with azithromycin and bronchodilators or glucocorticoid, and patients in the observation group were treated with combined therapy using montelukast and azithromycin, bronchodilators or glucocorticoid.

The application method for azithromycin: 0.2 g azithromycin was dissolved in 200 ml of 5% glucose solution and diluted into 1 mg/mL azithromycin solution for intravenous drip (once a day for 3 to 5 days). Based on patient's condition, it was changed to oral azithromycin (dosage=10 mg/kg·d twice per day). Patients received oral azithromycin for 4 consecutive days and stopped for 3 days (this was considered one course of treatment). The total treatment time was not less than 3 weeks. The asthma control was completed according to standard asthma prevention and control procedures. Based on the seriousness of the disease, the bronchodilators or glucocorticoid were used. In some cases, aerosol inhalation or intravenous drip methods were also used.

The application method for montelukast: for patients below 6 years old, the dosage was 4 mg and for patients above 6 years old, the dosage was 5 mg. Montelukast was taken once a day before sleep every night for 4 consecutive weeks.

Observation Indexes and Test Methods

The lung function indexes were measured before treatment and three weeks after treatment in both groups. These indexes included tidal volume (V-T), t-PTEF/t-E%, MTIF/MTEF, and TEF25/PTEF%. Children lung function monitor (Jaeger GmbH, Wurzburg, Germany) was used to test the tidal expiratory flow-volume loop. Patients were conscious and coordinative; the nasal cavity was tightly clamped. Tests were conducted for 2 consecutive times and the average values were recorded.

T lymphocyte subpopulation ($CD4^+$, $CD8^+$ and $CD4^+/CD8^+$) level, cytokines levels (IL-10, IL-17 and TGF-β), and positive rate of serum mycoplasma pneumoniae lgG and lgM in both groups before treatment and three weeks after treatment were compared. Fasting peripheral venous blood was collected (5 ml) and samples were centrifuged at 3000 g for 15 min and then transferred to -20°C.

FCM (Beckman Coulter, Fullerton, CA, USA) was used for selecting and counting T lymphocyte subpopulation. ELISA was used to measure the cytokines levels and the serum level of mycoplasma pneumoniae MPlgG and lgM. Kits were purchased from Sigma-Aldrich (St. Louis, MO, USA). The full-automatic ELISA was purchased from Multiskan FC Company (Waltham, MA, USA). We strictly followed the instructions provided by the kits' manufacturers. The reference value for MPnlgG was 10 to 15 kU/L, and < 17 kU/L for lgM. lgG>15 kU/L and lgM>17 kU/L indicated positive.

Asthma control rate and recurrence rate were calculated during the follow-up visits after 6 months and values were compared between groups. Asthma control rate meant that no asthma attack occurred within the 6 months, and the recurrence

Table I. Comparison of lung function indexes.

	V-T /kg(ml/kg)		t-PTEF/t-E (%)		MTIF/MTEF		TEF25/PTEF (%)	
Group	Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention
Observation group	8.2±1.2	11.5±1.8	32.6±10.3	45.7±9.6	0.8±0.3	1.3±0.5	52.4±12.5	77.5±9.3
Control group	8.3±1.3	9.8±1.7	33.5±12.4	41.6±8.4	0.8±0.2	1.1±0.4	53.6±13.6	72.6±8.8
t	0.216	5.216	0.326	5.032	0.562	5.324	0.526	5.327
p	0.758	0.036	0.752	0.038	0.741	0.033	0.634	0.031

rate meant that at least one acute attack occurred within 6 months.

Statistical Analysis

SPSS19.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Measurement data were expressed by the mean±standard deviation and comparison between groups was tested by independent sample t. Comparison within the group was tested by paired t and count data were expressed by rate and tested by χ^2. $p<0.05$ indicated that the difference was statistically significant.

Results

Lung Function Indexes

V-T, t-PTEF/t-E, MTIF/MTEF and TEF25/PTEF levels before intervention in the observation group were not significantly different compared with the control group ($p>0.05$). These levels increased in both groups after the intervention; however, the increase in the observation group was more significant. Differences were statistically significant ($p<0.05$) (Table I).

T Lymphocyte Subpopulation

CD4+, CD8+ and CD4+/CD8+ levels before intervention in the observation group were not significantly different compared with the control group ($p>0.05$). CD4+ and CD4+/CD8+ levels increased in both groups after the intervention; however, the increase in the observation group was more significant. Differences were statistically significant ($p<0.05$). After the intervention, CD8+ level decreased in both groups but the reduction was more evident in the observation group. Differences were statistically significant ($p<0.05$) (Table II).

IL-10, IL-17 and TGF-β

IL-10, IL-17 and TGF-β levels before intervention in the observation group were not significantly different compared with the control group ($p>0.05$). IL-10, IL-17 and TGF-β levels decreased in both groups after the intervention; however, the reduction was more intense in the observation group. Differences were statistically significant ($p<0.05$) (Table III).

Mycoplasma Pneumoniae IgG and IgM Positive Rate

IgG and IgM positive rates before intervention in the observation group were not significantly different compared with the control group (p>0.05). IgG and IgM positive rates decreased after the intervention, and the difference was statistically significant ($p<0.05$) (Table IV).

Asthma Control Rate and Recurrence Rate

Asthma control rate in the observation group was higher than that in the control group, while recurrence rate was lower in the observation

Table II. Comparison of T lymphocyte subpopulation (%).

	CD4+		CD8+		CD4+/ CD8+	
Group	Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention
Observation group	30.6±6.7	36.6±5.1	25.6±7.2	18.2±5.5	0.8±0.3	1.3±0.4
Control group	31.2±6.6	34.4±5.5	24.3±7.5	20.5±7.2	0.9±0.4	1.0±0.3
t	0.326	5.225	0.624	5.195	0.425	5.167
p	0.528	0.036	0.349	0.038	0.638	0.038

Table III. Comparison of cell factor levels.

	IL-10		IL-17		TGF-β	
Group	Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention
Observation group	123.6±42.5	63.5±23.2	86.2±26.3	37.8±12.5	32.3±8.2	12.5±5.7
Control group	120.8±53.2	88.4±27.3	83.4±30.2	54.5±16.7	31.7±8.6	16.8±5.3
t	0.865	5.637	0.564	6.225	0.427	5.467
p	0.234	0.020	0.637	0.013	0.632	0.025

Table IV. Comparison of *Mycoplasma pneumoniae* lgG and IgM positive rate.

		IgG		IgM	
Group	Case	Before intervention	After intervention	Before intervention	After intervention
Observation group	47	40 (85.1)	8 (17.0)	36 (76.6)	5 (10.6)
Control group	49	42 (85.7)	10 (20.4)	38 (77.6)	7 (14.3)
χ^2		0.007	0.181	0.012	0.292
p		0.933	0.671	0.911	0.589

group. The difference was statistically significant ($p<0.05$) (Table V).

Discussion

After *Mycoplasma pneumoniae* infection, epithelial cells in the airway swell while the collagen deposits increase in the airway and this may lead to *Mycoplasma pneumoniae*[7]. The clinical manifestation includes fever and irritable cough. Some sick children show symptoms like gasping and anhelation[8]. The azithromycin can effectively hinder the transpeptidation process in *Mycoplasma pneumoniae*, curb its proliferation, improve the inflammatory response of the lung and relieve clinical symptoms[9]. However, it cannot effectively relieve the asthma-like symptoms. *Mycoplasma pneumoniae* can trigger abnormal inflammatory responses in the airway, increase the airway hyperreactivity, and manifest the asthma-symptoms. It has been shown[10] that asthma attacks are closely related to the immunologic derangement, with the Th1/Th2 disorder, a decrease of CD4+ level and an increase of CD8+, leading to a tardive hypersensitivity.

Regular bronchodilators or glucocorticoid are not ideal tools for treating secondary asthma attacks caused by *Mycoplasma pneumoniae*[11]. Cough-type asthma can cause pulmonary hypofunction and prolong the course of the disease while making the treatment more difficult[12]. The lung function test can accurately evaluate the seriousness of the lung disease and provide effective assessment and prognosis. Our results revealed that V-T, t-PTEF/t-E, MTIF/MTEF and TEF25/PTEF levels in both groups increased after the intervention, with more significant improvement in the observation group. It was shown that joint treatment improved the lung function. Inflammatory and immunologic response plays an important role in the occurrence and development of pneumonia and asthma. It has been proved that IL-10, IL-17 and TGF-β can be produced, released and gathered in large quantities in airway epithelial cells, neutrophile granulocytes, eosinophilic granulocytes and mast cells. They can lead to inflammatory cascade reaction through multiple signal channels such as MAPK and P53[13].

The montelukast can stabilize the inflammatory cells' cytomembrane, and adjust the steady

Table V. Comparison of asthma control rate and recurrence rate during follow-up [case (%)].

Group	Case	Asthma control rate	Recurrence rate
Observation group	47	44 (93.6)	4 (8.5)
Control group	49	37 (73.5)	14 (28.6)
χ^2		5.966	6.337
p		0.015	0.012

state of inflammatory responses[14]. Our results demonstrated that $CD4^+$ and $CD4^+/CD8^+$ levels in both groups increased after the intervention, while the CD8+ level decreased, with more significant improvement in the observation group. The IL-10, IL-17 and TGF-β levels in both groups decreased after the intervention, with a stronger drop in the observation group. It was suggested that joint treatment improved the immunologic and inflammatory responses. The specificity test of mycoplasma lgG and lgM can provide important information about the diagnosis and treatment and help us to evaluate the immunologic reaction against the mycoplasma. We did not detect any significant difference between the positive rates of lgG and lgM in the observation group and the control group.

The asthma control rate in the observation group was meaningfully higher than that of the control group, while the recurrence rate in the observation group was lower than that of the control group. It was shown that the montelukast had a negligible effect on improving the immune function[15], but it was beneficial for controlling the symptoms of asthma and improving the prognosis[16].

Conclusions

The combined therapy using montelukast and azithromycin for treating the secondary asthma attacks in children mycoplasma pneumonia can relieve immunological and inflammatory reaction, improve the lung function, increase the asthma control rate and reduce the recurrence rate. Therefore, this treatment method is worth being promoted for clinical application.

Conflict of Interest

The authors declare no conflicts of interest.

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