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Original Article

Efficacy assessment and prognostic impact of combined serum IL-6 and IL-10 in the diagnosis of mycoplasma pneumonia in children

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Abstract: Objective: To determine the diagnostic efficacy and prognostic impact of combined serum interleukin-6 (IL-6) and interleukin-10 (IL-10) in children with mycoplasma pneumonia (MP). Methods: Children with MP (observation group, n=136) who were admitted to Qilu Children's Hospital of Shandong University from January 2017 to January 2018, and an equal number of healthy children (control group, n=136) were enrolled in this study. Serum IL-6 and IL-10 levels were measured by enzyme-linked immunosorbent assay before and after treatment, or on the day of physical examination in the case of the control subjects. Serum IL-6 and IL-10 levels in observation group before and after treatment, and those in control group were compared. Children with MP were grouped according to the presence or absence of fibrosis in their lungs and the severity of the disease. Serum concentrations of IL-6 and IL-10 of these sub-groups were also compared. The combined effect of serum IL-6 and IL-10 on the diagnosis of MP in children was analyzed using a ROC curve. Results: Baseline levels of serum IL-6 and IL-10 were higher in the observation group compared to control group (both $P<0.05$). However, IL-6 and IL-10 levels were significantly dropped in observation group after treatment (both $P<0.05$) and had no significant difference compared to those of the control group (both $P>0.05$). Furthermore, serum IL-6 and IL-10 levels were significantly higher in patients with fibrosis and severe disease compared to the non-fibrosis and non-severe groups respectively (all $P<0.05$). The sensitivity and specificity of combined serum IL-6 and IL-10 in the diagnosis of MP in children were greater than either factor alone, as per the ROC curve analysis (all $P<0.05$). Conclusion: Serum concentrations of IL-6 and IL-10 were significantly increased in children with MP. The combination of both cytokines had a greater diagnostic impact compared to the method of analyzing either one factor on MP in children. It can help in early diagnosis and assess the prognosis.

Keywords: Mycoplasma pneumonia in children, interleukin-6, interleukin-10, diagnosis, prognosis

Introduction

Mycoplasma pneumonia (MP), with a rising morbidity, is one of the most common types of pneumonia in children, accounting for 40% of all cases. It is the main reason for secondary pulmonary infection associated with multiple organ failure in children [1]. Epidemiological studies show that mycoplasma infection is the main cause of the disease. Due to the prolonged incubation period of this pathogen, the onset of symptoms is slow. In addition, symptoms, signs, imaging, and laboratory tests for MP are not specific. For immunocompromised children, the condition is severe at the later stages, along with a long disease course [2, 3]. The pathogenesis of MP in children is not yet clear at present. In contrast to the common belief that mycoplasma infections in the respiratory tract directly cause pneumonia, an increasing number of studies show that immunological injury may be the pathological basis of MP in children. In recent years, studies have shown that serum concentrations of interleukin-6 (IL-6) and interleukin-10 (IL-10) are significantly elevated in children with MP. The major reason is that mycoplasma infection stimulates antibody production and inflammatory cell infiltration, which results in the release of large amounts of cytokines. IL-6 and IL-10 are typical pro-inflammatory cytokines which play important roles in mediating immune injury [4, 5]. We

Table 1. Comparison of basic demographic data

Item	Male/female (case)	Average age (year)
Control group (n=136)	69/67	5.34±0.52
Observation group (n=136)	71/65	5.26±0.47
t/χ^2	0.059	1.331
P	0.808	0.184

Table 2. Comparison of the baseline serum concentrations of IL-6 and IL-10 between the control group and the observation group (pg/mL)

Item	Case	IL-6	IL-10
Control group	136	5.86±0.78	6.76±0.82
Observation group	136	21.47±1.26	20.59±1.74
t		122.800	83.850
P		<0.001	<0.001

Note: IL-6, interleukin-6; IL-10, interleukin-10.

therefore hypothesized that serum levels of IL-6 and IL-10 can be used as diagnostic indicators of MP in children, as well as in evaluating their prognosis. However, the status of serum IL-6 and IL-10 in mycoplasma pathogenesis in children is not yet clear. The aim of this study therefore was to analyze the diagnostic efficacy and prognostic impact of combined serum IL-6 and IL-10 in children with MP.

Materials and methods

General information

A total of 136 children with MP (observation group) who were admitted to Qilu Children's Hospital of Shandong University from January 2017 to January 2018 were enrolled for the study. The patients were divided into the fibrosis group (n=19) and non-fibrosis (n=117) group based on lung fibrosis. In addition, based on the severity of the disease, the patients were divided into the severe group (n=32) and the non-severe (n=104) group. The severity of the disease was assessed according to the clinical pulmonary infection score that includes seven indicators: body temperature, white blood cell count, tracheal secretions, oxygenation, chest radiography, the development of lung infiltrates, and tracheal aspirate culture. Each indicator is scored between 0 to 2 points, and the highest score is 12. Scores of 6 and above are considered as severe, while scores below 6 indicate a non-severe condition. Inclusion criteria of observation group were a positive diagnosis of MP, as indicated by the presence of serum antibodies and a positive chest X-ray.

The same number (n=136) of healthy children were enrolled as the control group. Inclusion criteria of control group were no history of infection and chronic disease in recent months; MP in control group was excluded by chest X-ray and serum antibody tests.

Exclusion criteria of the two groups: Patients with other respiratory diseases; those treated with antibiotics, immune modulators or inhibitors, or hormones before admission.

This study was approved by the Ethics Committee of Qilu Children's Hospital of Shandong University and the guardians of all included patients signed the informed consent.

Serum IL-6 and IL-10 determination

Three milliliters (3 mL) of cubital venous blood was drawn from each of the patients and healthy controls on the day of admission and after treatment, and on the day of the physical examination respectively. After anti-coagulation and centrifugation, the sera were separated and stored at -70°C till analysis. The levels of IL-6 (Shenzhen Jinzhun Biomedical Engineering Co., Ltd., China) and IL-10 (Shanghai Kanglang Biotechnology Co., Ltd., China) were measured by enzyme-linked immunosorbent assay, according to the manufacturers' instructions. Serum cytokine levels were compared between the patients and controls, and between the fibrosis/non-fibrosis and severe/non-severe patient sub-groups.

Statistical analysis

SPSS19.0 software was used for statistical analysis. Measurement data were expressed as the mean ± standard deviation ($\bar{x} \pm sd$) and compared between groups using t-test. Paired t-test was used for comparing pre-and post-treatment measurements. The enumeration data were expressed as the percentage and compared using the χ^2 test. The ROC curve was used to analyze the combined efficacy of serum IL-6 and IL-10 in the diagnosis of MP in children. The sensitivity, specificity and area under the

Table 3. Comparison of serum concentrations of IL-6 and IL-10 between pre-treatment and post-treatment in the observation group (pg/mL)

Time	Case	IL-6	IL-10
Pre-treatment	136	21.47±1.26	20.59±1.74
Post-treatment	136	6.04±0.89	6.94±0.91
t		67.550	27.398
P		<0.001	0.001

Note: IL-6, interleukin-6; IL-10, interleukin-10.

Table 4. Comparison of serum concentrations of IL-6 and IL-10 between the control group and the observation group after treatment (pg/mL)

Item	Case	IL-6	IL-10
Control group	136	5.86±0.78	6.76±0.82
Observation group	136	6.04±0.89	6.94±0.91
t		1.774	1.714
P		0.077	0.088

Note: IL-6, interleukin-6; IL-10, interleukin-10.

Table 5. Comparison of serum concentrations of IL-6 and IL-10 between the fibrosis group and the non-fibrosis group (pg/mL)

Item	Case	IL-6	IL-10
Fibrosis group	19	32.68±2.84	30.64±1.95
Non-fibrosis group	117	23.06±1.13	21.46±1.14
t		26.290	29.020
P		<0.001	<0.001

Note: IL-6, interleukin-6; IL-10, interleukin-10.

Table 6. Comparison of serum concentrations of IL-6 and IL-10 between the severe group and the non-severe group (pg/mL)

Item	Case	IL-6	IL-10
Severe group	32	30.64±1.57	26.34±1.92
Non-severe group	104	19.87±1.02	18.53±1.24
t		45.520	27.080
P		<0.001	<0.001

Note: IL-6, interleukin-6; IL-10, interleukin-10.

curve (AUC) was calculated. P<0.05 indicates statistically significant differences.

Results

Comparison of basic demographic data

There were 71 males and 65 females aged between 2 to 8, with the average age of 5.26±0.47 years in the observation group. In the control group, there were 69 males and 67 females aged between 3 to 10, with the average age of 5.34±0.52 years. The groups were not significantly different in terms of gender and age, and were comparable (both P>0.05). See **Table 1**.

Comparison of serum levels of IL-6 and IL-10

The baseline serum concentrations of IL-6 and IL-10 in the observation group were significantly higher compared to the control group (both P<0.001). See **Table 2**. Following treatment, both IL-6 (P<0.001) and IL-10 (P=0.001) levels significantly reduced in the observation group (**Table 3**) and were statistically comparable to those of the control group (all P>0.05). See **Table 4**. In addition, serum levels of IL-6 and IL-10 (both P<0.001) were significantly higher in the fibrosis group compared to the non-fibrosis group (**Table 5**), and in the patients with severe disease compared to those with non-severe disease (IL-6 and IL-10, both P<0.001). See **Table 6**.

Efficacy analysis of serum IL-6 and IL-10 combination in diagnosis

Serum IL-6 and IL-10 were used as test variables, and patients and healthy controls were used as effect variables. The threshold values of IL-6 and IL-10 were 7.0 pg/mL and 8.0 pg/mL respectively. The ROC curve analysis showed that the IL-6 and IL-10 combination had both higher sensitivity and specificity than those of either variable alone in the diagnosing MP in children (**Table 7**).

Discussion

Mycoplasma is a genus of cell wall-less prokaryotic microorganisms that are placed evolutionarily between viruses and bacteria. It is a common pathogen that causes respiratory infections, particularly among children, although the pathogenesis of MP in children is not clear. Studies have shown that mycoplasma infection can stimulate monocytes, lymphocytes, and macrophages to trigger cytokine-mediated inflammatory responses. MP in children is essentially an immunological reaction to the infection [6]. During the early stages of the infection, the immune system is activated by mycoplasma and produces multiple cyto-

Table 7. Efficacy analysis of serum IL-6 and IL-10 combination in diagnosis

Test variables	Sensitivity (%)	Specificity (%)	AUC	95% CI
IL-6	78.68	83.09	0.982	0.786-0.986
IL-10	84.56	76.47	0.902	0.803-0.904
IL-6 and IL-10 combination	92.65*,#	91.18*,#	0.886	0.812-0.937

Note: IL-6, interleukin-6; IL-10, interleukin-10; AUC, area under the curve; CI, confidence interval. Comparison of sensitivity between combination and IL-6, *χ^2=10.806, P=0.001; comparison of sensitivity between combination and IL-10, #χ^2=4.405, P=0.036; comparison of specificity between combination and IL-6, *χ^2=3.968, P=0.046; comparison of specificity between combination and IL-10, #χ^2=4.320, P=0.038.

kines to resist the mycoplasma invasion. However, excessive amounts of cytokines can result in immune-mediated injury and worsen the situation [7]. IL-6 and IL-10 are the major cytokines produced during the immune response, and the dynamic changes in their levels determine the development of MP in children [8]. Therefore, monitoring the concentrations of IL-6 and IL-10 can help monitor the course of the disease and treatment response.

IL-6 is produced by lymphocytes and other immune factors like antibodies. It has a wide range of biological activities and mainly mediates the inflammatory reaction against infections. Studies have shown IL-6 to be one of the core cytokines in the inflammatory response, as well as immune injury, in response to MP in children [9, 10]. IL-6 mediates inflammatory reactions either directly or indirectly by inducing the synthesis of acute-phase proteins [11, 12]. Guo et al. have shown that IL-6 can enhance the differentiation of T cells and promote the synthesis and secretion of autoantibodies, which explains the increased serum concentrations of IL-6 in children with MP [13]. IL-10 is essentially an anti-inflammatory factor that is produced by multiple immune cells. Large amounts of IL-10 can also be produced during inflammation, which can mitigate the latter and thus worsen infection [14-16]. There are only a few reports on the relationship between IL-10 and MP in children. We found that the serum concentrations of IL-6 and IL-10 were significantly higher in children with MP compared to the healthy controls. Our findings are consistent with those of Yan and Hassan et al., suggesting that serum concentrations of IL-6 and IL-10 are correlated with the progression of MP in children [17, 18]. The immune response to mycoplasma infection is similar to that against bacteria or viruses, with activation of immune cells that release multiple cytokines into the peripheral circulation [19]. Therefore, the early stages of mycoplasma infection would likely involve a balance between the pro-inflammatory and anti-inflammatory cytokines, which is consistent with the upregulation of both IL-6 and IL-10 seen in the patients with MP. Serum concentrations of both IL-6 and IL-10 significantly decreased after treatment and were restored to the levels in the control group. Therefore, the decrease in these cytokine levels and the balance between the pro-inflammatory and anti-inflammatory reaction can be important indicators of disease remission and good prognosis.

Pulmonary fibrosis is a pathological manifestation of severe conditions and a marker of poor prognosis in children with MP [20, 21]. Since the serum concentrations of IL-6 and IL-10 were significantly higher in patients with fibrosis or severe condition, they can help assess the severity of the disease. In addition, taking IL-6 and IL-10 as test variables and patients and healthy controls as effect variables, ROC curve analysis showed a greater diagnostic efficacy of the combination of IL-6 and IL-10, with higher sensitivity and specificity, compared to either cytokine alone. However, since the immunopathogenesis of MP is extremely complicated, a prospective controlled study is needed on a larger cohort to validate the current findings.

In conclusion, the serum concentrations of IL-6 and IL-10 are significantly increased in children with MP. Measurement of both cytokines can improve the diagnostic efficacy of MP in children and help in early detection. In addition, IL-6 and IL-10 levels in pediatric MP are clinically significant and can help assess prognosis and evaluate anti-mycoplasma therapy.

Disclosure of conflict of interest

None.

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