Original Article
Clinical significance of measuring MP-DNA, C-reactive protein, and inflammatory cytokines in children with mycoplasma pneumoniae pneumonia

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Abstract: Objective: To explore the clinical significance of detecting mycoplasma pneumoniae (MP)-DNA, C-reactive protein (CRP), interleukin-6 (IL-6), IL-8, and IL-10 in children with mycoplasma pneumoniae pneumonia (MPP). Methods: The data from 106 children who received treatment or underwent health examination in the Children’s Medical Center of Anhui Medical University from January 2021 to October 2022 were collected and analyzed retrospectively. The observation group (OG) consisted of 64 children with MPP, while the control group (CG) consisted of 42 healthy children. The levels of IL-6, IL-8, IL-10, CRP, and MP-DNA were compared between the two groups. The diagnostic value of MP-DNA in patients with MPP and its correlation with the levels of IL-6, IL-8, IL-10 and CRP were analyzed. Results: The level of MP-DNA in the OG was notably higher than that in the CG (P<0.05). Additionally, the levels of IL-6, IL-8, IL-10, CRP, and MP-DNA were compared between the two groups. The diagnostic value of MP-DNA in patients with MPP and its correlation with the levels of IL-6, IL-8, IL-10 and CRP were analyzed. The area under the curve of MP-DNA in diagnosing MPP was 0.979, with a specificity of 92.19% and a sensitivity of 97.62%. Conclusion: Indicators such as MP-DNA, IL-6, IL-8 are crucial in the development and progression of MPP, playing an important role in diagnosing and treating patients with MPP.

Keywords: Mycoplasma pneumoniae pneumonia, mycoplasma pneumoniae DNA, C-reactive protein, inflammatory cytokines

Introduction
Mycoplasma pneumoniae (MP) is a pathogen that falls between viruses and bacteria. It can cause acute respiratory infections and is one of the smallest microorganisms capable of independent survival [1]. MP is distinct from typical pneumonia caused by streptococcus pneumoniae, and it does not respond well to penicillin treatment. This is why it is often referred to as “primary atypical pneumonia” [2]. The pathological changes of MP are mainly interstitial pneumonia, occasionally accompanied by bronchopneumonia, with a higher incidence observed among children and adolescents [3]. MP is primarily transmitted from person to person through respiratory droplets, facilitating rapid local transmission within close communities and making it a major etiological agent for acquired pneumonia [4]. According to available data statistics, MP tends to occur in specific regions every 3-6 years, accounting for 10% to 25% incidence among community-acquired pneumonia (CAP) [5]. Studies have indicated that the highest incidence of MP is seen in individuals between the ages of 5 and 20, especially among children aged 5-9 and 10-15 [6]. At present, the specific pathogenesis of MP has not been clearly clarified. Some studies have revealed that the pathogenesis of MP is a complex and interconnected pathological process, which may be related to a variety of inflammatory mediators and cytokines, as well as the interaction in vivo and the role of pathogenic microorganisms on the host [7].

It is crucial to identify effective detection and treatment measures to improve prognosis, reduce mortality, and cure MP. MP-DNA refers to using DNA probes to detect the quantity of
mycoplasma in the body. This method is commonly used for the mycoplasma pneumoniae pneumonia (MPP) and is a potential method for early and rapid diagnosis of the disease [8]. Research [9] has revealed that MP-DNA has high sensitivity and can be used for early diagnosis and treatment of MP. In addition, interleukin (IL)-6 can regulate immune and inflammatory reactions, thus promoting body defense [10]. IL-8 plays a pivotal role in regulating both physiology and pathological processes within the human reproductive system [11]. IL-10 can participate in the biological regulation of immune cells, exerting significant influence on autoimmune diseases [12]. C-reactive protein (CRP) can be used to regulate the function of phagocytes and remove damaged or necrotic tissues and foreign pathogens [13]. Some scholars [14, 15] have revealed that inflammatory cytokines and CRP can also be used as predictive indicators for the diagnosis and treatment of CAP.

Therefore, this study aimed to provide reference for clinical diagnosis and treatment of MP by studying the expression and clinical significance of MP-DNA, CRP, IL-6, IL-8 and IL-10 in patients with MPP.

Methods and materials

Sample collection

Patient medical records of 120 children were retrieved and collected from the electronic medical record system of the Children’s Medical Center of Anhui Medical University. The children either received treatment or underwent health examination there from January 2021 to October 2022.

Inclusion and exclusion criteria

Inclusion criteria: Patients in the OG met the diagnostic criteria of MPP [16]; Individuals in the CG were healthy without any diseases; Patients aged between 1 and 14 years old; Patient who had complete clinical data.

Exclusion criteria: Children who suffered from other infectious diseases, mental disorder or severe heart, liver and kidney diseases; Patients who quit the treatment midway.

Sample screening

According to the inclusion and exclusion criteria, 106 individuals who met the requirements were included. A retrospective study was conducted using the medical records of the 106 patients. The observation group (OG) consisted of 64 children with MPP, while the control group (CG) consisted of 42 healthy children who underwent health examination. This research was ratified by the Medical Ethics Committee of the Children’s Medical Center of Anhui Medical University.

Index detection

Detection of serum inflammatory cytokines and CRP: Morning fasting venous blood (4 mL) was drawn from all the subjects and centrifuged at a speed of 3000 r/min for 10 min to separate the serum. The serum was then divided into two tubes and stored at low temperature for further testing. Serum IL-6, IL-8, and IL-10 were measured by radioimmunoassay, and serum CRP were detected by enzyme-linked immunoassay (Shanghai Enzyme-linked Biotechnology Co., Ltd., item number: mlsh-0789-1).

Detection of MP-DNA: Morning sputum samples were collected from the lungs of the subjects using a suction catheter after performing a gargling procedure. The sputum samples were then stored at low temperatures for subsequent testing. The level of MP-DNA was detected using ABI500 fluorescent quantitative polymerase chain reaction (PCR) and MP-DNA fluorescent quantitative kit. All the operating techniques were strictly implemented in accordance with the instructions.

Outcome measures

The levels of IL-6, IL-8, IL-10, CRP, and MP-DNA were compared between the two groups. The diagnostic value of MP-DNA for MPP and its correlation with the levels of IL-6, IL-8, IL-10, and CRP were analyzed.

Statistical methods

SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was utilized for data analysis. GraphPad Prism 8 was applied to visualize the data. The classified variables were analyzed using the chi-square. Measurement data in line with normal distribution were analyzed by t-test, independent sample t-test for inter-group comparison and paired t-test for intra-group comparison. Pearson’s correlation test was used to analyze the correlation between MP-DNA and the levels of IL-6,
Clinical significance of biochemical markers for mycoplasma pneumoniae pneumonia

Table 1. Comparison of baseline data

<table>
<thead>
<tr>
<th>Factors</th>
<th>Control group (n=42)</th>
<th>Observation group (n=64)</th>
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<td>17</td>
<td></td>
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</table>

The statistical significance was determined with a threshold of P<0.05.

Results

Comparison of baseline data

There was no statistical difference in age, gender, height, weight, place of residence, and ethnicity between the two groups (P>0.05) (Table 1).

Comparison of MP-DNA

The MP-DNA level of the patients in the OG was obviously higher than that of the subjects in the CG (P<0.05) (Figure 1).

Comparison of serum inflammatory cytokines and CRP

Comparing the levels of serum inflammatory cytokines and CRP, the results revealed that the levels of IL-6, IL-8, IL-10 and CRP in the OG were significantly higher than those in the CG (P<0.05) (Figure 2).

Correlation between MP-DNA and serum inflammatory cytokines, CRP

Pearson test was conducted on the correlation between MP-DNA and IL-6, IL-8, IL-10, and CRP in both groups. The results showed that MP-DNA was positively correlated with these four indexes (P<0.05) (Figure 3).

Clinical value of MP-DNA in the diagnosis of MP

ROC curve was introduced to understand the clinical value of MP-DNA in the diagnosis of patients with MP. The results revealed that the area under the curve (AUC) of MP-DNA in the diagnosis of MP was 0.979, with a specificity of 92.19% and a sensitivity of 97.62% (Table 2; Figure 4).

Discussion

MP is currently one of the main pathogens causing respiratory tract infections in children [17]. It is a highly contagious infection that tends to spread rapidly within close-knit com-
Clinical significance of biochemical markers for mycoplasma pneumoniae pneumonia

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Figure 2. Comparison of serum inflammatory cytokines and CRP. A. Comparison of serum IL-6 level. B. Comparison of serum IL-8 level. C. Comparison of serum IL-10 level. D. Comparison of serum CRP level. Note: **** means P<0.0001. IL-6: interleukin-6, IL-8: interleukin-8, IL-10: interleukin-10, CRP: C-reactive protein.

Community, such as in kindergartens. Over the years, there has been an increasing incidence of mycoplasma infection due to societal development and changes in living habits. Research indicates that MP accounts for 20% of children’s CAP in China and 50% of CAP in children over five years old [18]. This disease can occur at any age and can lead to more serious diseases without timely treatment. If a patient is infected with MP, they generally exhibit flu-like symptoms such as runny nose, nasal congestion, fever, cough, and headache. The fever can vary in severity, with 39°C in some patients. Additionally, after 2-3 days, affected individuals may develop noticeable respiratory symptoms, such as paroxysmal irritating cough [19]. Current research has highlighted the strong association between MP in children and asthma. If the progression of MP is not effectively managed in a timely manner, the probability of developing asthma can be as high as 50%. As the respiratory system of children is not fully developed, it is very likely to cause lung function impairment after being infected with mycoplasma [20]. In addition to respiratory symptoms, some children may experience complications such as aseptic inflammation of the nervous system, or muscle and joint pain. In severe cases, MP can even pose a life-threatening risk. Therefore, it is of great significance to identify suitable diagnostic indexes for the diagnosis and treatment of MP.

Studies have shown that MP-DNA can better reflect the infection in children, and it is closely related to the severity and prognosis of the disease [21]. The detection of MP-DNA through fluorescence quantitative PCR offers various advantages, including high sensitivity and specificity. MP-DNA contributes to the early diagnosis of diseases, which can be highly beneficial in clinical settings. In this study, we used the fluorescence quantitative PCR technology to detect the MP-DNA level of the subjects. The results showed that the MP-DNA level of the patients in OG was significantly higher than that of the subjects in CG, suggesting that there was a large amount of replication of mycoplasma in the bodies of the affected children, which caused serious damage to the respiratory system. The results indicate that the level of MP-DNA can timely reflect the disease status of affected children, thereby improving the diagnostic rate of MP, enabling timely treatment, shortening the disease duration, and reducing their suffering, which is similar to the research results of Gu et al. [9]. Inflammatory cytokines are related to the development of MP and participate in inflammatory reaction, immune reaction and injury [22, 23]. Research by Zhang et al. [24]
Clinical significance of biochemical markers for mycoplasma pneumoniae pneumonia

Figure 3. Correlation of MP-DNA with serum inflammatory cytokines and CRP. A. Correlation between MP-DNA and IL-6. B. Correlation between MP-DNA and IL-8. C. Correlation between MP-DNA and IL-10. D. Correlation between MP-DNA and CRP. Note: IL-6: interleukin-6, IL-8: interleukin-8, IL-10: interleukin-10, CRP: C-reactive protein, MP-DNA: mycoplasma pneumoniae DNA.

Table 2. Clinical value of MP-DNA in the diagnosis of mycoplasma pneumoniae

<table>
<thead>
<tr>
<th>Factors</th>
<th>MP-DNA</th>
</tr>
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<tbody>
<tr>
<td>AUC</td>
<td>0.979</td>
</tr>
<tr>
<td>Confidence interval</td>
<td>0.953-1.000</td>
</tr>
<tr>
<td>Specificity</td>
<td>92.19</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>97.62</td>
</tr>
<tr>
<td>Youden index</td>
<td>89.81</td>
</tr>
<tr>
<td>Cut-off</td>
<td>5.545</td>
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</table>

Note: AUC: area under the curve, MP-DNA: mycoplasma pneumoniae DNA.

Figure 4. ROC curve of MP-DNA in the diagnosis of mycoplasma pneumonia. Note: ROC: Receiver operating characteristic; MP-DNA: mycoplasma pneumoniae DNA.

revealed that CRP, IL-6 and other related indicators were important predictors MP in children, and detection of these indicators could be helpful for early identification of MPP.

In this study, we analyzed and compared the levels of serum inflammatory cytokines in the subjects. The results showed that the levels of IL-6, IL-8, IL-10, and CRP in the OG were significantly higher than those in CG, indicating that the levels of inflammatory cytokines increased significantly in children infected with MP. The results suggest that cytokines such as IL-6 and inflammatory mediators are closely related to the pathogenesis of MP.

In addition, the correlation between MP-DNA and the levels of IL-6, IL-8, IL-10, and CRP was analyzed. It was found that MP-DNA was positively correlated with these four indexes, indicating that MP-DNA had a certain correlation with the levels of IL-6, IL-8, IL-10, and CRP. Our results are similar to the findings of Fang et al. [25]. By detecting the level of IL-6 and other indicators during the disease, the development and prognosis of the disease can be reflected, so they can be used as an auxiliary diagnostic index. Furthermore, we analyzed the diagnostic value of MP-DNA using ROC curve. The results showed that the AUC of MP-DNA in the diagnosis of MP was 0.979, and the specificity and sensitivity were 92.19% and 97.62%, respectively. It is suggested that MP-DNA can be used as an important reference index for the diagnosis and treatment of MPP.

In this study, we have revealed the diagnostic value and clinical significance of MP-DNA and serum inflammatory cytokines in children with MPP. However, there are still some limitations in this study. Firstly, this is a retrospective study with a limited sample size, which may potentially result in less conclusive experimental findings. Secondly, patients could not be followed up in this study, so the long-term effect and
Clinical significance of biochemical markers for mycoplasma pneumoniae pneumonia

prognosis of patients could not be compared and observed. Therefore, we hope to carry out more experiments in the future to improve our research conclusions.

To sum up, MP-DNA, IL-6, IL-8 and other indicators are crucial in the development and progression of MPP. These indicators have been found to play an important role in diagnosing and treating patients with MPP. Therefore, they have a high diagnostic value and are of great significance for future clinical diagnosis and treatment.

Disclosure of conflict of interest

None.

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References

Clinical significance of biochemical markers for mycoplasma pneumoniae pneumonia