Prevalence and clinical characteristics of septicemia in children with *Mycoplasma pneumoniae* pneumonia

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Abstract

Background: *Mycoplasma pneumoniae* (MP) pneumonia in children can be challenging to treat, and the impact of MP blood infection is unclear. The present study aims to determine the prevalence and clinical characteristics of MP septicemia among pediatric patients.

Methods: Children hospitalized at our center for MP pneumonia between October 2017 and June 2018 were included. Healthy controls visiting our outpatient clinic for regular physical examinations were also enrolled. MP was detected by real-time polymerase chain reaction (qPCR) analysis of plasma and peripheral blood mononuclear cell (PBMC) samples.

Results: Sixty-one children with MP pneumonia and 30 healthy children were included. Among children with MP infection, 31 (50.8%) were positive for MP by qPCR (19 in plasma samples, 8 in PBMC samples, and 4 in both). All healthy controls were negative for MP by qPCR.

Conclusions: The prevalence of MP septicemia in children with MP pneumonia is moderate. However, detection of MP in blood samples may have limited clinical value for guiding treatment.

Introduction

Mycoplasma pneumoniae (MP) is a common pathogen that causes respiratory infection in most patients; infection develops into pneumonia in 3% to 13% of patients.[1]

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In children, MP is a common etiological agent of community-acquired pneumonia (CAP) and is responsible for 10% to 40% of CAP cases.[2] Fortunately, most cases of respiratory infection by MP are self-limiting. However, during recent decades, several factors have made the treatment of MP pneumonia in children more challenging. Examples include increasing trends in the drug resistance of MP as well as in the proportion of patients experiencing severe MP pneumonia.

Most research on MP infection has focused primarily on the clinical characteristics of MP pneumonia, and relatively little is known regarding MP bloodstream infection. In 1974, a MP strain was first isolated from the blood by Naftalin et al.[3] In a recent study by Scapani et al.,[4] another MP strain was isolated from a blood culture. These findings showed that MP bloodstream infection is common in clinical practice, and that bloodstream infection may play a role in the development of extrapulmonary MP-associated diseases. In a previous study, Daxboeck et al.[5] found that MP was present in the bloodstream of a substantial proportion of patients with MP infection. However, the authors used polymerase chain reaction (PCR) from serum as the only diagnostic tool for MP infection; alternative techniques and sampling may improve the detection of MP. The clinical characteristics of MP bloodstream infection remain uncertain, and further investigations are necessary.

In addition to lung injury, MP infection can also have extrapulmonary manifestations. It is thought that MP can disseminate to other organs through the bloodstream, resulting in injuries to other organs. This may explain why MP bloodstream infection is more common in patients with extrapulmonary MP infection than in patients with pulmonary MP infection.[6] In addition, distinct immune responses may also be associated with MP bloodstream infection. For example, an increased level of inter-α-trypsin inhibitor heavy chain H4 in serum was reported in a patient with MP bloodstream infection.[7]

Recently, PCR has been applied for the detection of MP bloodstream infection.[6] In the present study, real-time PCR (qPCR) detection of MP was performed using plasma and peripheral blood mononuclear cell (PBMC) samples to estimate the prevalence of MP bloodstream infection in children with MP pneumonia. The clinical data of children with or without MP bloodstream infection were compared to identify any meaningful differences associated with bloodstream infection.

Patients and methods

Patients

The study conformed to the principles outlined in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the First Hospital of Jilin University (approval number: 2018-463). Written informed consent was obtained from patients' parents or guardians.

Between October 2017 and June 2018, children (aged <15 years) admitted to our hospital and hospitalized for MP pneumonia were included in the study. Healthy controls were selected from individuals visiting our outpatient clinic for regular physical examinations without a reported medical history. Suspected MP pneumonia was defined by a positive IgM titer (>1:40) or an increase in the IgG titer of >4 times the initial value.[8,9] The exclusion criteria were chronic respiratory tract infection and macrolide therapy within the previous 2 weeks.

MP IgG assay

A serum sample was obtained from all patients. Anti-MP antibody in serum was

detected using a passive agglutination assay (Serodia-MYCO II, Fuji Rebio Ltd., Tokyo, Japan) according to the manufacturer's instructions. The test was judged as positive if a specimen showed a negative (−) result with unsensitized particles (1:20 final dilution) but a positive (+) result with sensitized particles (1:40 final dilution). The test was judged as negative if a specimen showing a negative (−) result with sensitized particles (1:40 final dilution).

PCR

To detect MP in PBMC and plasma samples, samples were prepared as follows. For plasma, whole ethylenediaminetetraacetic acid-treated blood samples were collected and centrifuged at $2000 \times g$ for 10 minutes. Subsequently, plasma was centrifuged at $13,500 \times g$ for 10 minutes. After centrifugation, the supernatant was discarded and the remaining sample was used for DNA extraction. PBMCs were isolated by gradient separation using Ficoll-Histopaque (10771; Sigma-Aldrich, St. Louis, MO, USA). After separation, the cells were washed with phosphate-buffered saline three times and pelleted by centrifugation for DNA extraction. qPCR was performed using a commercial kit for MP DNA (PCR Fluorescence Probing; Daan, Guangzhou, China). The product is designed for use with sputum and throat swab samples and is not for use with blood samples.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, USA). Continuous variables were presented as means (standard deviations) or medians (25th and 75th percentiles). Categorical variables were presented as frequencies (percentages). Differences between groups were assessed using the Student's t test or Mann–Whitney U test for normally distributed and non-normally distributed continuous variables, respectively. Differences between categorical variables were assessed using Chi-square or Fisher's exact tests. Values of $P < 0.05$ were considered statistically significant.

Results

Baseline characteristics

Between October 2017 and June 2018, a total of 61 children (aged <15 years) with MP pneumonia met the inclusion criteria and were enrolled. The mean (±standard deviation) age was 6.20 ± 3.74 years, and 57.4% (n = 35) of patients were boys. In addition, 30 healthy children with no previous medical history were selected as controls (male, n = 23; female, n = 7; mean age 4.56 ± 3.82 years). Baseline characteristics did not differ significantly between the two groups. Table 1 and Supplementary Table 1 show the clinical characteristics of pediatric patients MP pneumonia with and without MP bloodstream infection.

Prevalence of MP bloodstream infection in pediatric patients with MP pneumonia

The prevalence of MP bloodstream infection was estimated based on qPCR detection of MP in PBMC and plasma samples from all patients with MP pneumonia (n = 61). Samples from 31 patients (50.8%) were positive; MP was detected in the PBMCs of eight patients, in the plasma of 19 cases, and in both the plasma and PBMCs of four patients. All healthy controls were negative for MP by qPCR analysis of PBMC and plasma samples.

Comparison of clinical characteristics between groups

The clinical characteristics of pediatric patients with MP pneumonia with and

Table 1. Clinical characteristics of pediatric patients with *Mycoplasma pneumoniae* (MP) pneumonia with or without MP bloodstream infection

	MP pneumonia cases (N = 61)					MP qPCR (−) (n = 30)	P (+ vs. −)	Total	Healthy controls (n = 30)
	MP qPCR (+) (n = 31)								
	Plasma	PBMCs	Both	P	Total				
N	19 (61.3%)	8 (25.8%)	4 (12.9%)		31 (50.8%)	30		61	30
Sex (male:female)	12:7	4:4	2:2	0.770	18:13	17:13	0.912	35:26[b]	23:7
Age, years	5.53 ± 3.82	8.78 ± 4.35	8.5 ± 1.29	0.095	6.76 ± 3.97	5.63 ± 3.45	0.244	6.20 ± 3.74[b]	4.56 ± 3.82
Underlying diseases/symptoms									
Wheeze	7 (36.8%)	2 (25%)	1	0.708	10 (31.25%)	4 (13.3%)	0.079	14	
Laboratory examinations									
Throat swab MP qPCR (+)	5 (22.7%)	2 (9.1%)	3 (13.6%)	0.309	10 (45.5%)	12 (54.5%)	0.601	22	
Throat swab MP qPCR (−)	5 (26.3%)	3 (15.8%)	1 (5.3%)		9 (47.4%)	10 (52.6%)		19	
LDH, U/L	352.84 ± 73.55[a]	278.63 ± 89.39	266.75 ± 43.42	0.030	322.58 ± 82.52	353.14 ± 99.56	0.203		

Data are shown as mean ± standard deviation or n (%).

[a]P < 0.05 compared with PBMC group or both PBMC and plasma group.

[b]P > 0.05 compared with healthy control group.

LDH, lactate dehydrogenase; MP, *Mycoplasma pneumoniae*; PBMC, peripheral blood mononuclear cell; qPCR, real-time polymerase chain reaction; U, units.

without bloodstream infection were compared. Age, sex, fever, hospitalization time, symptoms, and laboratory tests showed no significant differences between the two groups.

qPCR detection of the presence of MP in throat swabs was performed for 41 patients and was positive for 22 of these patients. Among children with a positive throat swab for MP, there was a trend toward positive PCR results for MP in plasma only (rather than positive results in PBMCs or in both PBMCs and plasma). Moreover, the mean age of children with a positive plasma MP qPCR result showed a lower trend than the mean ages of children with positive results from PBMCs or both PBMCs and plasma.

Notably, wheezing was more common in cases of MP pneumonia with MP bloodstream infection than in cases without MP bloodstream infection (P < 0.05). In addition, associations between plasma MP load and clinical characteristics (fever, time between symptom onset and admission, time to fever clearance, hospitalization time, and time from fever onset to discharge) were also investigated. No significant differences were found.

Discussion

In this study, the prevalence of MP bloodstream infection in pediatric patients with MP pneumonia was 50.8%. Moreover, the clinical characteristics associated with bloodstream infection were assessed, but no variables were associated with MP bloodstream infection. Thus, detection of MP bloodstream infection may have limited clinical value in the management of pediatric patients with MP pneumonia.

MP strains were isolated from the blood by Naftalin et al. and Scapini et al. [3,4] Culture methods are generally unsuccessful in isolating MP, limiting their use in MP-related diagnostics. The low MP burden in

blood makes MP isolation even more difficult. Fortunately, MP detection in blood by PCR was demonstrated by Narita et al. in 1996.[6] The authors reported that the prevalence of MP bloodstream infection among patients with pediatric MP pneumonia was very low at only 4% (1/25). This result is inconsistent with our data. The discrepancy may relate to differences in methods used for MP detection between studies (PCR vs qPCR). In a recent study of adult MP infection, Daxboeck et al.[5] compared the effectiveness of conventional PCR and qPCR for MP detection in blood. The authors found that PCR returned a negative result for all patients (n = 29), whereas qPCR provided a positive result for 52% (n = 15) of patients. Interestingly, the qPCR results in this population were similar to the findings of our study. These data showed that MP bloodstream infection in patients with MP pneumonia may be more common than previously thought. In addition, healthy controls were also investigated and showed negative qPCR results for MP in all cases. These results confirm that MP DNA was absent from the blood collected from children without MP infection.

Among children with MP pneumonia, 31 (50.8%) were positive for MP bloodstream infection by qPCR (19 in plasma, 8 in PBMCs, and 4 in both). These results indicate that MP can be present in plasma and blood cells. Consistently, in vitro experiments have showed that MP can adhere to human red blood cells.[10,11] Recently, Deas et al.[12] discovered interactions between MP and human blood red cells via scanning and transmission electron microscopy experiments. Hence, a potential mechanism was proposed: MP adheres to red blood cells and then disseminates from the lungs to other sites.[13] Usually, the pathogenesis of MP infection is thought to involve deleterious effects on the host cell epithelium. Although intracellular growth and replication have been described for MP in vitro,

this process has not been shown to occur during natural infection.[14,15] Our findings suggest that MP also could adhere to PBMCs.

The reasons underlying differential detection of MP in plasma and PBMCs in patients with pediatric MP pneumonia remain unclear. It is not merely a case of low DNA burden in samples. One potential explanation may be that, at the initiation of infection, MP adheres to cells and can be detected first in PBMC samples by qPCR. Subsequently, the pathogen is released into the blood, allowing for later detection in plasma by qPCR. The distribution of MP between cells and plasma changes with the clinical progression of MP pneumonia. Two key points from the results of our study support this explanation. First, children with a positive result of qPCR for MP in PBMCs had a shorter period between symptom onset and qPCR than did those with a positive result of qPCR for MP in plasma (7.5 ± 8.16 days vs 9.84 ± 6.18 days). Second, occasionally, PBMC and plasma qPCR analyses were performed at different times for the same child (admission and 2 weeks after hospitalization). In some children, PBMC qPCR analysis was positive for MP at the admission (but negative in plasma), while 2 weeks later, plasma qPCR for MP became positive. Moreover, the prevalence of MP bloodstream infection was similar in children with a positive throat swab qPCR result and those with a negative result. This finding showed that the time required for MP clearance differed between the throat and blood, with MP emerging in the throat first and then disseminating to the blood. Likewise, after treatment, MP disappears from the throat first and then disappears from the blood. Although a false-negative result for MP may occur from qPCR analysis of blood, qPCR remains a useful tool for evaluating the MP burden in samples.[16]

The symptoms caused by MP infection are not limited to the lungs.[17–20] Three mechanisms may explain the extrapulmonary manifestations of MP infection.[21] First, lipoproteins localized at the membrane of MP could produce an inflammatory response with cytokine release. Second, immune modulation, including autoimmune responses induced through cross-reactivity between bacterial cell components and human cells, may lead to indirect injury. Third, vascular occlusion may be induced by the bacterium and manifest as vasculitis and/or thrombosis with or without a systemic hypercoagulable state. Based on the above-mentioned mechanisms, it can be concluded that the blood dissemination of MP plays an important role in the pathogenesis of MP infection. For example, Stamm et al.[22] reported a fatal case of acute disseminated encephalomyelitis followed MP pneumonia that was confirmed by subsequent autopsy findings.

Prior to conducting the present study, we hypothesized that children with MP bloodstream infection would have more severe infection than those without bloodstream infection. However, no significant differences were found in most clinical characteristics between the two groups, including hospitalization time, fever, underlying diseases, and laboratory results. Children with a positive result of plasma qPCR analysis for MP were younger than those with positive results from PBMC RT-PCR analysis or both plasma and PBMC qPCR. Overall, our results indicate that the bloodstream infection did not make the MP infection more serious, according to the comparison of clinical characteristics between groups. Although the presence of MP in blood has been shown in many studies,[23,24] the clinical influence of the infection remains unclear. In addition, Narita et al.[6] showed that MP bloodstream infection is more common in patients extrapulmonary MP infection than in patients with pulmonary MP infection.

Hence, further analysis is required to investigate the mechanism of MP bloodstream infection and its role in the pathogenesis of extrapulmonary MP infection.

Conclusions

MP bloodstream infection is common in pediatric MP pneumonia with a moderate prevalence. The clinical characteristics of patients with MP pneumonia and MP bloodstream infection were not different from those of patients with MP pneumonia without MP bloodstream infection. This implies that detection of MP blood infection may have limited clinical value. However, more research is needed to investigate the characteristics of MP bloodstream infection.

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Supplemental Material

Supplemental material for this article is available online.

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