

Original Article

Variation in serum antibody titers in children with community-acquired *Mycoplasma pneumoniae pneumonia*

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Abstract: *Mycoplasma pneumoniae pneumonia* (MPP) is one of the most common primary atypical pneumonias caused by *Mycoplasma pneumoniae* (MP). Its early diagnosis is difficult. In this study, we examined the variation in serum antibody titers in children with MP infection and explored its early diagnostic value. This prospective case series involved 134 children with MP infection. Serum antibody detection was performed with a commercially available particle agglutination test (SerodiaMyco II; Fujirebio, Tokyo, Japan). The chi-squared test was used to identify significant associations between disease duration and the rate of serum antibody positivity. Linear correlation and simple linear regression were used to describe the relationship between the mixed antibody titer and disease duration. Positivity rates (serum antibody titer $\geq 1:160$) at 4-7 days, 8-14 days, and 15-21 days were 41.7%, 91.6%, and 100%, respectively, with significant differences among timepoints. $\log(\text{IgA} + \text{IgM} + \text{IgG})^{-1}$ was correlated positively with disease duration ($r = 0.832$, $P < 0.01$). Of 85 children with community-acquired MPP showing a fourfold or greater increase in serum antibody titer at a 2-week interval, 75 (88.24%) children showed such an increase at a 1-week interval. The present study demonstrated that the serum antibody titer is related closely to the duration of MP infection, and suggest that a 2-week interval is not needed for the diagnosis of MP infection using paired serum tests. This finding may have great diagnostic value, enabling short-term retesting for the diagnosis of MP infection.

Keywords: Community-acquired infection, *Mycoplasma pneumoniae*, serum antibody

Introduction

Mycoplasma pneumoniae pneumonia (MPP) is one of the most common primary atypical pneumonias caused by *Mycoplasma pneumoniae* (MP), which is also a primarily etiological agent in community-acquired pneumonia (CAP) in children [1, 2]. The clinical symptoms and radiological findings of children with MPP and those with pneumonia caused by other pathogens do not differ significantly [3]. In other words, the clinical manifestation is non-specific in the diagnosis of MP infection, which must be supported by microbiological findings. Although rapid tests, such as polymerase chain reaction (PCR) and direct antigen detection based on monoclonal antibodies, are more sensitive and specific [4, 5], they are often too expensive or technologically intensive for general use in clinical practice. Recently, serological assays were found to be crucial tools for the diagnosis of MP infection, with equal sensitivity

as the detection of acute infection with culture and comparable sensitivity to PCR [6, 7]. The SerodiaMyco II particle agglutination (PA) test (Fujirebio, Tokyo, Japan), in particular, is used in numerous laboratories because of its practical advantages [8].

In this study, we examined the variation in serum antibody titers at different time points after disease onset in children with CA-MPP using a PA test, an easily performed test with greater sensitivity and specificity for the detection of serum antibodies than other serological assays. We aimed to provide a basis for the early diagnosis of MP infection.

Materials and methods

Study population

This prospective study involving children with confirmed CA-MPP was conducted at the Aff-

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Table 1. Distribution of serum antibody titers in children with community-acquired *Mycoplasma pneumoniae* pneumonia

Titer	4-7 d [% (n specimens)]	8-14 d [% (n specimens)]	15-21 d [% (n specimens)]
(-)	17.143 (12)	0.000 (0)	0.000 (0)
1:40	11.429 (8)	2.797 (4)	0.000 (0)
1:80	24.286 (17)	5.594 (8)	0.000 (0)
1:160	20.000 (14)	11.189 (16)	0.000 (0)
1:320	15.714 (11)	10.490 (15)	7.692 (5)
1:640	5.714 (4)	9.790 (14)	10.769 (7)
1:1280	1.429 (1)	13.287 (19)	6.154 (4)
1:2560	0.000 (0)	11.189 (16)	15.385 (10)
1:5120	2.857 (2)	15.385 (22)	12.308 (8)
1:10,240	1.429 (1)	12.587 (18)	21.538 (14)
1:20,480	0.000 (0)	7.692 (11)	26.154 (17)
Total	100 (70)	100 (43)	100 (65)

Table 2. Serum antibody positivity rates in children with community-acquired *Mycoplasma pneumoniae* pneumonia

Disease phase	< 1:160	≥ 1:160	Total	Positivity rate	P
4-7 d	33	37	70	47.1%	< 0.001
8-14 d	12	131	142	91.6%	0.016
15-21 d	0	65	55	100%	< 0.001

iliated Hospital of Qingdao University, China, from January 2013 to November 2014. The hospital's ethics committee approved the study, and informed consent was obtained from the guardians of all patients. Children were enrolled according to the following criterion: simultaneous diagnosis with CAP [8, 9] and MP infection based on PA test positivity (single titer ≥ 1:160 or > 4-fold increase or decrease in paired titers).

Serum testing

Serum was collected from all participants in the early (1-7 days after the onset of disease symptoms), middle (8-14 days after symptom onset), and late (15-21 days after symptom onset) disease phases. Serum samples were analyzed using the SerodiaMyco II gelatin PA test (product no. YZB/JAP 1885-2009; Fujirebio), which measures mixed antibody [immunoglobulin (Ig) G, IgA, and IgM] titers of MP based on the principle of hemagglutination of these antibodies to MP, according to the manufactur-

er's instructions [10]. Erythrocytes are replaced by latex particles to avoid non-specific reactions. Serum specimens were inactivated at 56°C for 30 min. Rigid U-well microtiter plates (supplied with the kits) were soaked in detergent solution overnight and then rinsed thoroughly under running tap water, washed with distilled water, and dried. Using the serum diluent supplied, 25- μ l serum specimens were double diluted to 1:10-1:10,240. Sensitized and unsensitized lyophilized gelatin particles were suspended in diluent. Drops of the unsensitized particle suspension (25 μ l) were added to the 1:10 serum dilutions to yield a final dilution of 1:20, and 25- μ l drops of the sensitized particle suspension were added to the remaining wells to yield final dilutions of 1:40-1:20,480. The plates were shaken for 30 s and then covered and left undisturbed on a level surface at room temperature for 3 h (or overnight).

The test was initially calibrated using the control serum dilution series. Each batch of tests included control wells containing 25 μ l diluent and 25 μ l particle suspensions and dilutions of a reactive control serum of known titer, supplied with the kit. Buttons or compact, smooth rings of particles in the bottoms of the wells were read as negative agglutination patterns, and more extensive rings were considered to be positive patterns. A fourfold or greater increase in titers of paired sera and single serum titers ≥ 1:160 were regarded as positive results.

Statistical analysis

The chi-squared test was used to identify significant associations between disease duration and the rate of serum antibody positivity. Linear correlation and simple linear regression were used to describe the relationship between the mixed antibody titer and disease duration. All analyses were performed using SPSS software (ver. 17.0; SPSS Inc., Chicago, IL, USA). All *P* values were three tailed, and *P* < 0.05 was considered to be statistically significant.

Results

A total of 134 children (56 boys, 78 girls) aged 6 months-14 years with confirmed CA-MPP were enrolled in the study. We analyzed 278 serum samples collected from these 134 children (single sera from 49 children, multiple or

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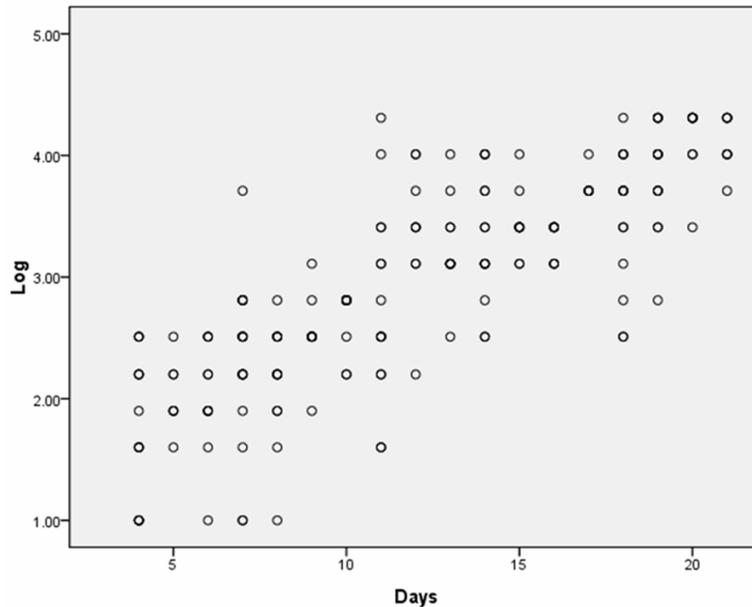


Figure 1. Correlation between serum antibody titer [$\log(\text{IgM} + \text{IgA} + \text{IgG})^{-1}$] and disease duration after onset (4-21 days) in children with community-acquired *Mycoplasma pneumoniae pneumoniae*.

paired sera from 85 children; **Table 1**). Seventy specimens were collected in the early disease phase, 143 were collected in the middle phase, and 65 specimens were collected in the late disease phase.

Positivity rates, based on titers $\geq 1:160$, in the early, middle, and late phases were 47.1% (33/70), 91.6% (131/143), and 100% (65/65), respectively (**Table 2**). The seroprevalence of serum antibodies differed significantly among phases (overall: $\chi^2 = 82.152$, $P < 0.001$; early vs. middle: $\chi^2 = 52.459$, $P < 0.001$; middle vs. late: $\chi^2 = 5.788$, $P = 0.016$; early vs. late: $\chi^2 = 47.329$, $P < 0.001$).

The serum antibody titer [$\log(\text{IgM} + \text{IgA} + \text{IgG})^{-1}$] increased with the duration of disease and was correlated positively with this duration ($r = 0.832$, $P < 0.01$; **Figure 1**). The regression equation was $Y = 10^{1.251 + 0.14X}$, where Y represents the serum antibody titer and X represents disease duration ($F = 471.702$, $P < 0.001$).

Fourfold increases in paired serum antibody titers were detected in 85 children at an interval of 2 weeks and in 75 of these children at an interval of 1 week (**Table 3**). Sixteen cases showed fourfold increases, 13 cases showed

eightfold increases, and 46 cases showed increases of 16-fold or more.

Discussion

Recently, great emphasis has been placed on the role of atypical pathogens in children with CAP. About 31.3% of CAP cases have been found to be triggered by atypical pathogens, most commonly MP infection [11]. IgM usually appears within 1 week of initial MP infection and is always implicated in acute infection in the clinical setting. Upon reinfection, however, the IgM response can be absent [12]. IgG generally appears 2 weeks after IgM. It may be considered to be the most important parameter in MP serology, as serological diagnosis is confirmed upon the appearance or significant augmentation of IgG in serum between the acute and convalescent phases, at an interval of 2 weeks. IgA is produced in the early phase of the disease, and levels can peak quickly and decrease more rapidly than those of IgM and IgG. The SerodiaMyco II gelatin PA test enables semi-quantitative detection of mixed antibodies (including IgM, IgG, and IgA) and has greater specificity and sensitivity than other serology assays [13]. However, the paired serum assay has limited practical value for the early diagnosis of MP infection in the clinical setting because of the need to obtain samples at 2-week intervals and the close relationship between an increased single serum antibody titer and disease duration. Accordingly, examination of the variation in serum antibody titers in children with CA-MPP is of paramount significance to determine the optimal timing for the early diagnosis of MP infection.

Consequently, a significantly increase in serum antibody titer can be used to diagnose early MP infection. Katsushima et al. [14] and Youn et al. [15] found that serum antibody titers increased with the onset of MP infection. Beersma et al. [16] reported that serum antibody titers were low in the early symptom onset phase, and increased with positivity rates in the 2 weeks

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Table 3. Variation in serum antibody titers of 75 children with community-acquired *Mycoplasma pneumoniae* pneumonia

Initial Titer	Titer after 1-week interval (n children)										Total
	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10,240	1:20,480	
(-)	1			3	1	3		1			9
1:40		2		1	1	1	2				7
1:80				3	1	2	2	2	2		12
1:160						5	5	4	4	1	19
1:320						5	4	6	3	1	19
1:640							4	1	2	1	8
1:1280								1			1
Total							75				

after symptom onset (7-25% 1-6 days after symptom onset, 31-69% at 7-15 days, 33-87% at ≥ 16 days), in adults with CA-MPP. However, they reported a high rate of false-negative results of serum analyses. In our study, serum antibodies were detected on the 4th day after CA-MPP onset, and the positivity rate was correlated positively with disease duration in children. Our findings are not in complete agreement with the results reported by Beersma et al., perhaps due to differences in study population. Nevertheless, the seroprevalence of serum antibody ($\geq 1:160$) was low at 4-7 days, which may lead to misdiagnosis of MP infection based solely on a single serum titer. Seroprevalence increased rapidly at 8-14 days, and titers $\geq 1:160$ had greater diagnostic value, but we could not rule out the possibility of MP infection entirely due to negativity in 8.4% of cases. We observed 100% seroprevalence at 15-21 days. These findings indicate that positivity of a single serum antibody titer is not sufficient for the diagnosis of current MP infection. The diagnosis of current MP infection should be based on a combination of clinical findings and results of paired serum testing.

The gold standard for the diagnosis of MP infection based on serological detection is a fourfold or greater increase in IgG antibody titer in paired serum samples obtained at an interval of at least 2 weeks. Sobieszcańska et al. [17] reported that the evaluation of acute- and convalescent-phase sera is necessary for accurate interpretation of serological testing results. Yamazaki et al. [18] reported that paired serum testing is clinically useful for the diagnosis of MP infection, and that it can result in the correction of missed diagnosis based on single

serum testing. However, paired serum testing for the early diagnosis of MP infection has little clinical or practical significance, due to the long interval (2 weeks) required. Thus, attention has recently been given to the identification of a way to diagnose MP infection as early as possible based on the variation in antibody titers in paired serum samples in the clinical setting. Liu et al. [19] reported a fourfold or greater titer increase in children with MP infection at 8-18 days after symptom onset, with 29.1% of children showing antibody negativity (titer $< 1:40$) at a mean of 5.16 days (range, 4-7 days) after symptom onset. In our study, 88.24% of children with CA-MPP showing fourfold or greater titer increases at a 2-week interval also showed such increases at a 1-week interval; this finding suggests that the diagnosis of MP infection can be based on paired serum testing at a 1-week interval, which has crucial diagnostic value. Moreover, the duration of seroconversion (negative to positive) has been found to be about 1 week after the first examination [20]. Lee et al. [16] observed seroconversion in approximately 30% of children with MPP; similarly, we observed seroconversion from the first detection in 36% (27/75) of children showing 1-week titer increases. Thus, the false-negative rate may be higher for the first serum detection, which would adversely affect timely diagnosis and treatment in the clinical setting. Retesting at a 1-week interval may decrease the rate of missed diagnosis.

This study has several limitations. The sample was small and consisted entirely of patients at a single tertiary children's hospital; these patients likely had more severe infections, which may have affected serum antibody titers in several ways.

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In summary, serum antibody titers were related closely to disease duration in children with CA-MPP, and a 2-week interval is not required for the diagnosis of MP infection using this criterion. This finding has great value for the early diagnosis of MP infection. Serological tests are also used for the diagnosis of various respiratory infections, such as viral infections [21, 22]. Further research is needed to determine whether a 1-week interval is sufficient for paired serum antibody testing in the context of these conditions.

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Disclosure of conflict of interest

None.

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