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ORIGINAL ARTICLE

Association of *Mycoplasma pneumoniae* antibody titer level with clinical severity of pneumonia in children

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ABSTRACT

The objectives is to establish a positive correlation between the serology titer of Mycoplasma pneumoniae and the severity of pneumonia, with an emphasis on severity of disease presentation between two different age groups. The research included 110 children admitted at the Tianjin Medical University General Hospital, Tianjin, China, from May 2010 to October 2010. This study included disease history, clinical examination findings, laboratory investigations including Mycoplasma pneumoniae antibody titer (MP-IgM) level as well as imaging, and duration of in-patient treatment. The results of the parameters were also compared between two age groups: children <5 years old and ≥5 years old. Seventy-six percent of the patients who had severe presentation of the disease had a high titer of MP-IgM and 63% of them were older than five years. Platelet level seems to be a promising indicator of progression of disease. Leukocytosis was present in only 4.5% of the 110 children. The C-reactive protein was significantly raised in those older than five years of age. A significant correlation has been found between the severity of disease and Mycoplasma pneumoniae serology titer level in children suffering from Mycoplasma pneumoniae pneumonia.

***Keywords**: Mycoplasma pneumoniae; MP-IgM; pneumonia in children; thrombocytosis*

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Introduction

Mycoplasma pneumoniae (*M. pneumoniae*) is an important causative organism of respiratory tract infections. Isolated for the first time in the early 1940s, seven decades later it is still one of the commonest agents to cause community-acquired pneumonia. *M. pneumoniae* infections cause an array of signs and symptoms, ranging from asymptomatic ones to quite severe and even fatal pneumonia with extrapulmonary manifestations. In this study, we will focus on the clinical manifestations, laboratory studies and imaging techniques, with more emphasis on how the immunological response can be related to the severity of disease.

Materials and methods

The research was performed in Tianjin, China, from the pediatric ward at the Tianjin Medical University General Hospital. The patients were prospectively followed and observed from the day of admission until the day of discharge from the hospital. For a better follow-up of the evolution of disease in relation to the signs and symptoms, only admitted patients were included in this study.

Diagnostic parameters evaluated

The diagnostic parameters involved the present disease history, laboratory investigations, imaging findings, clinical improvement during the course of treatment, extrapulmonary manifestations and number of days of admission.

Patient recruitment and procedures

One hundred and ten patients admitted between May 2016 and October 2016 were chosen at random to be part of this study. All of them were

confirmed cases of *Mycoplasma pneumoniae* (MP) pneumonia by serology titer of MP-IgM (1:≥80) and symptoms of pneumonia. A small amount of patients with MP-IgM titer of 1:40 (n = 15) who were clinically diagnosed with MP pneumonia were also included in the study due to their classical clinical manifestations and response to specific treatments of MP pneumonia.

A set of criteria were taken into consideration to classify the severity of disease into mild and severe. Fever, extrapulmonary changes such as abnormal values of liver function test LFT, CKMB and clinical manifestations such as hepatomegaly, rashes, meningitis, encephalitis, gastroenteritis and carditis, as well as the number of days the children were admitted, the number of courses of intravenous antibiotics and the imaging techniques results were included into the assessment. The chest radiographic patterns of patients on admission with MP pneumonia were divided into two groups. Patients with increased nodular densities along the bronchial trees and interstitial pattern on the unilateral or bilateral lung fields were designated in the mild description. Patients with distinctive subsegmental, segmental or lobar consolidation, unilateral or bilateral pleural effusion, atelectasis and consolidation visible on X-ray films and computerized tomography (CT) scans were included in the severe description.

The criteria were marked as zero (0) if the patient had a fever of less than 10 days since the beginning of the disease, no extra-pulmonary manifestations, the patient had a single 10-day course of intravenous antibiotics during admission, the findings on the chest X-ray fell into the mild category and the patient was discharged in less than 12 days of admission.

On the other hand, the criteria were marked as one (1) if the fever was present more than 10 days since the beginning of the disease, extrapulmonary findings were present, the patients needed more than 10-day course of intravenous antibiotics during admission, the imaging results fell into the severe description and the patient was discharged after a minimum of 12 days or more. A total score of ≥3 classified the patient as a severe case. For a more detailed report, the 110 patients were divided into two groups according to age: <5 years of age (48 patients) and ≥5 years of age (62 patients), and the clinical and laboratory data as well as severity of disease were compared. The criteria are summarized in **Table 1** below.

The total score for each patient was obtained by adding the scores of all the criteria. A final score of <3 will categorise the severity of disease as mild and if a total score of ≥ 3 was obtained, the pneumonia was considered as severe.

Table 1. Table of criteria

Criteria	Score 0	Score 1
Fever (F)	≤10 days	>10 days
Extrapulmonary manifestation (EM)	absent	present
X-ray pattern (X-ray)	interstitial	localised
IV antibiotics (IV Abx)	≤10 days	>10 days
Days discharged (Dd)	<12 days	≥12 days

Analysis

The data collected, namely age, sex, *Mycoplasma pneumoniae* antibody serology titer IgM, leukocyte count, platelet count, neutrophil and lymphocyte counts and the C-reactive protein level, were included in the analyses. Imaging findings, the duration of fever and the severity of disease were among the important data to be included. The analyses were done using Statistical Package for the Social Science (SPSS version 17.0). The goal was to mainly support that there is a positive correlation between the serology antibody titer of MP-IgM and the severity of pneumonia. Continuous variables and descriptive statistics are reported as the mean ± standard deviation using the independent sample *t*-test, the one-way analysis of variation (ANOVA) for continuous variables and the Pearson's correlation analysis to find statistical significance between the parameters.

Results

A total number of 110 patients participated in the study. All of them were admitted patients with a confirmed diagnosis of *Mycoplasma pneumoniae* pneumonia. Forty-eight percent out of those patients were female. The study revealed that 44% of the patients were less than five years old and the mean age for the group of patients was 6 ± 4 years old. The ratio of female to male was 1:1.1. The majority of the followed patients had their main complaints as having cough and fever, with the exception of seven of them among whom four had fever only at the beginning of the disease and the rest presented with cough only. No statistical significant correlation was found between MP-IgM titer level and age and gender, with $P > 0.05$ in both analyses. The average number of days of fever was 10 ± 5 days and a statistically significant correlation was noted with the severity of disease ($r = 0.388$, $P < 0.01$). It has been

observed that the duration of fever tends to be longer in most cases of high MP-IgM titers.

The serology test for MP-IgM was done at the end of the first week (on the 7th day) or within the second week of disease manifestation, confirming the diagnosis. Twelve cases were revealed as being seroconverters, out of which nine were older than five years of age and all of them had a high titer. In the routine complete blood count (CBC), we have observed that in only 4.5% of cases, the leukocytes increased. A decrease in lymphocytes was noted in 83% of the cases, while neutrophils showed an increase in its level in 54% of patients. Age-related reference values were taken into consideration for the leukocyte and lymphocyte counts.

However, an interesting finding noted during this study was that the platelets were raised in 55% of the cases, and 77% of the patients with raised thrombocytes level had a high serology titer level. The C-reactive protein (CRP) increased in 66% (n = 73/110) of the children admitted, out of whom 75% (54/73) had a quite high serum titer of MP-IgM. Statistically it has been found that lymphocytes, CRP and the Mycoplasma titer values had a significant correlation with the severity of disease. The leukocyte, platelet and neutrophil values showed no statistical correlation with MP pneumonia severity. It has been noted that lymphocytes level had a statistically significant negative correlation with disease severity.

Table 2 summarizes the results obtained from the statistical analysis performed to find any significant difference in the laboratory parameters between mild and severe presentations of MP pneumonia.

Table 2. Statistical significance of laboratory parameters between mild and severe degree of disease

Laboratory findings	Severity of disease		P^*
	Mild (n = 64)	Severe (n = 46)	
Leukocytes (x 10^9/L)	8.8 ± 3.6	8.3 ± 3.1	0.429
Neutrophils (%)	60.5 ± 13	65.0 ± 10	0.069
Lymphocytes (%)	33 ± 13	27 ± 9	0.004
Thrombocytes (%)	293 ± 84	315 ± 112	0.259
CRP (mg/dl)	1.4 ± 1.3	2.4 ± 2.2	0.006

* $P < 0.05$ for statistical significance

As shown in the table, leukocytes and thrombocytes as well as neutrophils revealed no significant correlation with severity of MP pneumonia. Lymphocytes level revealed a negative correlation with disease severity with r = -0.257 and $P = 0.007$, leading to suggest that lymphocyte count decreases as the disease gets more severe. CRP is positively correlated with severity of pneumonia. Though no significant correlation was found between severity of disease and thrombocytes level, an analysis of the latter with MP-IgM titer level showed that there was a significant positive correlation between the two parameters with a P value of 0.028, suggesting that as MP-IgM titer level rises so does the level of platelets.

The results in patients less than five-years-old

A general expression of results of the study has been elucidated above. However, some differences with the clinical and laboratory findings have been noticed in the younger ones. Though a correlation between a high Mycoplasma pneumoniae antibody titer and severity of disease was found, it was also noted that disease criteria have a tendency to be milder in the clinical presentation in younger patients, in this case younger than five years old.

Table 3 summarizes statistical analyses of laboratory parameters between mild and severe degree of disease in children <5 years old. No statistical significant difference was found between mild and severe degree of disease in relation to any of the above parameters in this age group.

Table 3. Statistical analyses of laboratory parameters between mild and severe degree of disease in children <5 years old

Laboratory findings	Severity of disease		P^*
	Mild (n = 32)	Severe (n = 16)	
Leukocytes (x 10^9/L)	10 ± 4.3	9.3 ± 3.4	0.513
Neutrophils (%)	56 ± 15	60 ± 11	0.398
Lymphocytes (%)	37 ± 14	32 ± 10	0.186
Thrombocytes (%)	314 ± 80	341 ±1 11	0.398
CRP (mg/dl)	1.1 ± 1.2	1.2 ± 0.8	0.839

* $P < 0.05$ for statistical significant difference

Table 4 summarizes the statistical correlations of the parameters, between the age groups <5 years old and ≥5 years old.

A negative correlation was obtained with lymphocytes, and CRP was positively correlated. It has been revealed that no correlation was found

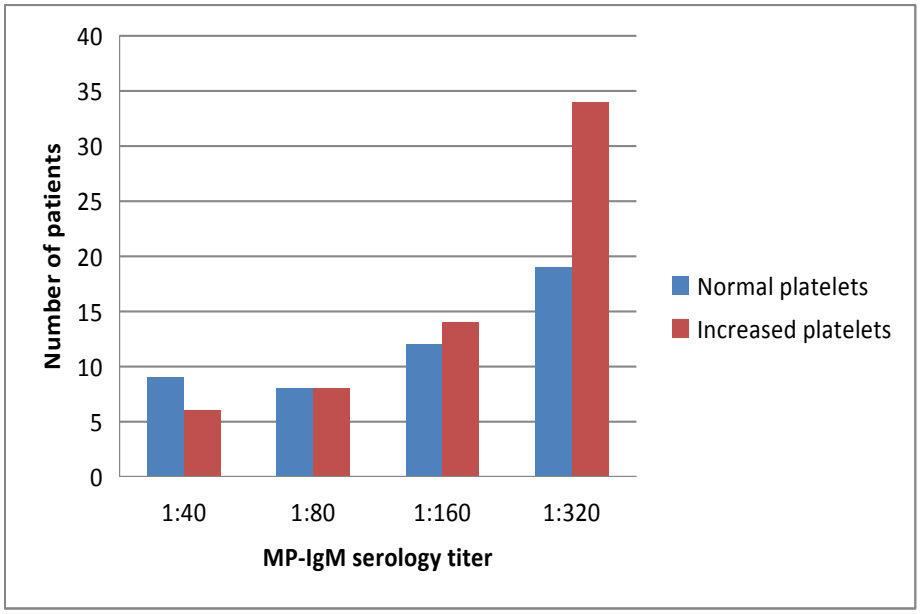

Figure 1. Trend of thrombocytes count with MP-IgM titer levels

Table 4. Statistical correlations and significance of the parameters, between the age groups <5 years old and ≥5 years old

	Children <5 years old		Children ≥5 years old	
	r	***p***	**r**	***p***
Fever	0.307	0.034	0.435	< 0.001†
Leukocytes	-0.097	0.513	0.045	0.727
Lymphocytes	-0.194	0.186	-0.257	0.044
Thrombocytes	0.140	0.343	0.152	0.238
Neutrophils	0.125	0.398	0.141	0.274
CRP	0.030	0.839	0.311	0.014

r = Pearson's correlation coefficient, $P < 0.05$ for statistical significance
† $P < 0.01$ for statistical significance

between any of the laboratory parameters and the severity of disease in the age of <5 years old group as compared to the older children.

After having considered all the set of criteria, a positive statistically significant correlation (r = 0.496, $P < 0.05$) exist between severity of disease and IgM titer of *Mycoplasma pneumoniae* and a graphic representation of disease severity distribution in relation to the MP-IgM titer level is shown in **Figure 2**.

Discussion

Although it is being reported that *Mycoplasma pneumoniae* pneumonia is less common in children who are under five years of age, in the recent years we have noticed a change in the epidemiology in age distribution. Recent studies have concluded that children under five years, including infants, are more commonly affected by *Mycoplasma pneumoniae*[1–7]. From the data collected in our ward itself, out of the 504 cases admitted from January 2010 to December 2010, 33.7% were younger than five years old. The number of patients admitted started to peak from the month of July 2010 onwards.

Some changes in the incidence of *M. pneumoniae* infections, in which high number of cases occurred between epidemics without a return to lower endemic levels, have led to speculations as to the reasons for this occurrence. Jacobs[8] in 2002 suggested that the availability of new information on virulence factors and the P1 adhesin, along with the deciphering of the complete genome, might enhance the understanding of why these changes have occurred. However, a recent study performed by a Swedish team, Nilsson *et al*., supported that clinical severity of *Mycoplasma pneumoniae* infections is associated with bacterial load rather than the MP genotype[9].

As already mentioned earlier, the severity of disease and disease presentation in affected children younger than five years old tend to be less severe and different than those older, according to the clinical observations done during this study as well as according to some others[4,5,10]. Patients with higher titers of antibodies took longer time to recover from fever, cough and abnormal lungs findings.

The C-reactive protein test has revealed to be a very good indicator of the seriousness of disease. Even though it is non-specific to *Mycoplasma*

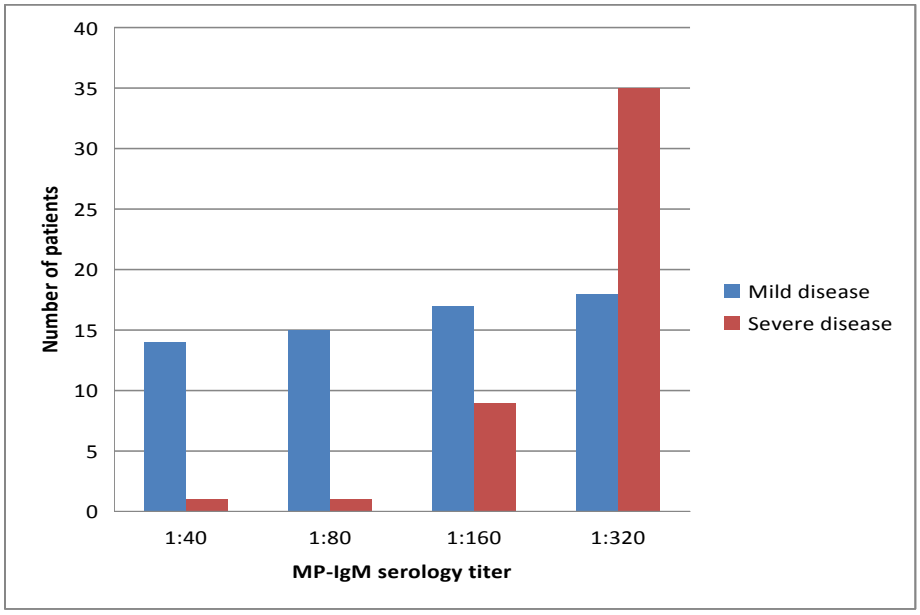

Figure 2. Trend of thrombocytes count with MP-IgM titer levels

pneumoniae infection, it is needless to say that the CRP parameter has long proven itself to be an excellent asset in detection of acute inflammatory process. Seventy-five percent of the children with a high CRP value had high MP titer level (IgM ≥1:160). Only 15% of those having titer of MP-IgM ≥1:320 did not have an increase in CRP levels. Forty-eight out of the 73 patients who had a raised CRP were older than five years. The leukocytes did not appear to bring any significant interest to the severity of disease[11]. In general, no correlation to their values was made with the titer of MP-IgM.

One of the most interesting findings concerning laboratory results in this study was the astonishing rise in the platelet counts noted in the patients followed. Thrombocytosis has been observed in most cases during the second week of disease, corresponding with the increasing of MP-IgM titer. Where the level was very high as well as where the disease was more serious, the platelets were also raised (>600 x 10^9/L) in some in-patient cases observed. Recent research mentioned that platelets may have an anti-bacterial effect[4,5,12]. It has been proposed that, as platelets rapidly accumulate at sites of infection, they may play similar "watch guards" roles to that of traditional immune cells such as macrophages, mast cells and dendritic cells. Platelet surface has effects on adaptive immunity, including accelerating dendritic cell maturation, stimulating IgG production by B cells and enhancing T cell activity. Their adherence to and aggregation around bacteria may promote bacterial clearance; indeed, activated platelets may hold bacteria, thereby removing them from the circulation[13,14].

Though the therapeutic implications of interactions between bacteria and platelets are still being explored, some specialists think that the anti-bacterial potential of platelets could be utilized by selectively releasing, or mimicking, thrombocidins which are microbicidal proteins found in the human blood platelets[15].

Among the patients selected for this study, it has been observed that 11% of them were IgM seronegative at presentation of MP pneumonia on the first serology test for IgM done at the end of the first week of disease and they eventually turned out to be seroconverters on the second test usually done after one week. Absence of diagnostic IgM antibodies in the early stage of systemic infections has been well documented in previous studies of adults and children with MP infections[16,17]. Interestingly, there were more seroconverters in the group of those older than five years of age.

Conclusion

The clinical aspect and tendencies observed in this study generally highly suggest that a higher MP-IgM titer leads to more severe presentation of disease as well as more serious laboratory and imaging changes including CRP, platelets, chest X-rays and CT scans. However, the clinical phenotype of MP pneumonia differs with age, with a longer period of fever, higher CRP, and more severe pulmonary lesions observed in older children.

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