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Short Communication

Application value of time of appearance of *Mycoplasma pneumoniae* IgM antibodies in rapid diagnosis

Xia Wang [1], Jingjing Han [2], Lijiao He [2] and Ling Gao [2,*]

[1] Department of Clinical Laboratory Medicine, Nanjing Red Cross Hospital，Nanjing City, Jiangsu Province, China

[2] Department of Clinical Laboratory Medicine, Children's Hospital of Nanjing Medical University, Nanjing City,Jiangsu Province, China

* Corresponding author, e-mail:gaolingtony@163.com

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Abstract: This retrospective study examines the optimal timing for *Mycoplasma pneumoniae* (MP) IgM detection in children with clinical symptoms such as cough and fever, and evaluates the diagnostic value of MP-IgM levels with routine blood tests. Clinical data from 149 children with MP IgM positivity were analysed, focusing on symptom onset times and changes in IgM levels, white blood cell (WBC), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) at varying symptom durations. Coughing appeared as early as 24 hr after infection, with fever following 12 hr later. The median cough duration was 7 days, with 54.4% of children testing IgM positive within this period, while fever was present in 59.1% of cases. IgM, WBC, NLR and PLR levels were significantly higher than those in the control group in children with cough durations of 8-15 days, and prolonged fever (>3 days) correlated with higher IgM and PLR levels. It is concluded that for children with suspected MP infection, quantitative MP-IgM testing combined with routine blood parameters (WBC, NLR, PLR) is recommended for rapid diagnosis, particularly in those with cough persisting >7 days or fever lasting >3 days.

Keywords: pediatric *Mycoplasma pneumoniae* pneumonia, rapid diagnosis, quantitative *Mycoplasma pneumoniae* IgM, white blood cell, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

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INTRODUCTION

Mycoplasma pneumoniae (MP) is one of the main pathogens causing respiratory tract infections globally. It is an important pathogen in pediatric community-acquired pneumonia, accounting for approximately 20-40% of cases and up to 50% during epidemics [1, 2]. Understanding the epidemiological characteristics of MP infection is crucial for clinical diagnosis and prevention. This disease typically occurs sporadically, with regional outbreaks every 3-7 years lasting 1-2 years each time, and can occur in all seasons throughout the year [3]. Severe MP pneumonia presents challenges in clinical treatment due to direct respiratory damage and potential damage to multiple organs and systems. Therefore, early diagnosis and treatment of MP infection in children are crucial. Current expert consensus recommends combining pathogen and serological indicators for laboratory testing for MP. In clinical practice serological testing plays a vital role as a rapid and convenient screening tool in the diagnosis of MP infection. Outpatient serum IgM antibody rapid testing for children is usually completed within 2 hr, significantly improving detection efficiency.

However, due to individual differences in the time of IgM antibody production after MP infection, some children may have a weak antibody response or low antibody levels upon reinfection, leading to false-negative results. In addition, clinical doctors have different understandings of the time of IgM antibody production after MP infection, which often raises questions about the reports of mycoplasma testing. For example, in clinical practice there are sometimes cases where the serum IgM antibody test shows negative titers (COI values) in children highly suspected of MP infection, which raises concern about the timing of IgM antibody production.

Therefore, this study aims to explore the importance of the time of MP-IgM antibody production in rapid outpatient diagnosis. By retrospectively analysing the time of onset of clinical symptoms (such as coughing and fever) in children who tested positive for *M. pneumoniae* IgM, the optimal timing of MP-IgM detection can be determined. Additionally, based on previous analyses of routine blood test results in our laboratory, it was found that white blood cell (WBC) count, neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) levels were generally elevated in MP-infected children. Thus, this study also evaluates the diagnostic value of combining quantitative MP-IgM testing with routine blood tests (WBC, NLR, PLR).

MATERIALS AND METHODS

Inclusion and Exclusion Criteria for Clinical Data

This study collected the clinical data of 149 children (IgM positive) diagnosed with *M. pneumoniae* infection at the Affiliated Children's Hospital of Nanjing Medical University from January 2024 to June 2024. The data included the time of onset of clinical symptoms such as coughing and fever, the highest temperature during fever, MP-IgM antibody quantitative test results and routine blood test results. The inclusion criteria were: 1) age between 28 days and 18 years; 2) MP-IgM positive; 3) complete data on clinical symptoms such as coughing, fever and peak

temperature. The exclusion criteria were: 1) isolated MP-IgG antibody positive; 2) incomplete clinical symptom data.

Retrospective Analysis of Clinical Data

Among the 149 children, there were 73 males and 76 females, with ages ranging 5 months - 13 years and an average of 4.8 years. The control group consisted of 20 children who underwent health examinations at our hospital during the same period, consisting of 10 males and 10 females, aged 6 months - 11 years (average 4.3 years).

Detection Methods and Result Determination Criteria

Two mL of venous blood was collected from the subjects and serum was separated. Serum MP-IgM antibody levels were measured using chemiluminescence immunoassay with the MP IgM test kit (Shenzhen YHLO Biotech Co., China), following the instructions. A cut-off index (COI) of less than 0.9 was considered non-reactive/negative, COI between 0.9-1.1 was considered weakly positive, and COI equal to or greater than 1.1 was considered reactive/positive. Routine blood tests were performed using the Mindray BC7500 automated haematology analyser with corresponding reagents.

Statistical Analysis

Statistical analysis of the data was performed using SPSS 24.0 and Excel statistical software. The Shapiro-Wilk test confirmed that all data had a skewed distribution and the data were visualised using percentiles (P2.5, P97.5) and medians.

For the IgM, WBC, NLR and PLR data of children with different symptoms, between-group comparisons were performed using the nonparametric Shapiro-Wilk test, and pairwise comparisons were performed using the Dunn-Bonferroni test. A p-value less than 0.05 was considered statistically significant.

RESULTS

Distribution of Clinical Symptom Onset in MP Patients

All 149 patients with cough symptoms had the earliest onset of coughing resulting in positive IgM detection after 1 day and the latest after 90 days. The average duration was 13.6 days with a median of 7 days. Among the 149 patients, 88 children experienced fever symptoms, with the earliest onset resulting in positive IgM detection after 0.5 day and the latest after 30 days. The average duration was 4.8 days, with a median of 4 days. Among the 88 febrile patients, the peak temperature ranged up to 40.7°C, with a mean of 39.1°C and a median of 39.1°C (Table 1).

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Table 1. Distribution of cough, fever onset and peak temperature in MP-IgM positive patients

Clinical Symptom	No. of Cases	P2.5	P5	P25	P50	P75	P95	P97.5
Cough (days)	149	2	2	5	7	14	45	90
Fever (days)	88	1	1	2	4	7	10	14
Peak temperature (°C)	88	37.6	37.9	38.7	39.1	39.6	40.2	40.7

Note: P2.5-P97.5 indicate 2.5th-97.5th percentiles respectively.

The numbers of cases with fever and cough in children with MP infection are shown in Figure 1. For example, among the patients, 22 (14.8%) tested positive for IgM antibodies within 1-3 days of cough onset, 6 without fever, 11 with fever lasting 1-3 days and 5 with fever lasting more than 3 days, while 55 patients (39.6%) tested positive for IgM antibodies within 4-7 days of cough onset, 19 without fever, 13 with fever lasting 1-3 days, and 23 with fever lasting more than 3 days.

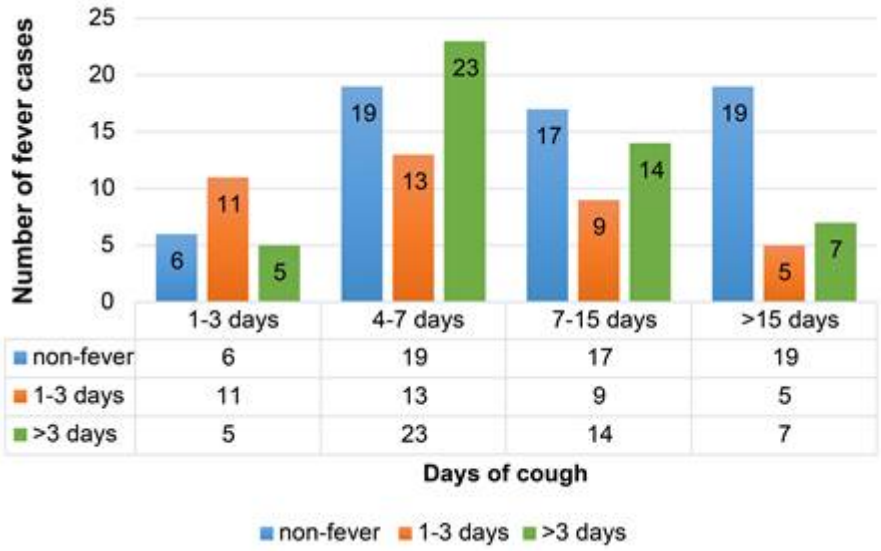

Figure 1. Distribution of cough and fever duration in pediatric patients with MP infection

Association Between IgM Antibody Concentration, WBC, NLR, PLR and Duration of Cough

The results indicate a significant correlation between the concentration of IgM antibodies, WBC count, NLR, PLR and the duration of cough in the children (Table 2). Specifically, the concentration of MP-IgM in the group displaying cough symptoms for 8-15 days is markedly higher compared to those in the group with cough durations of 1-3 days, 4-7 days and more than 15 days. Children whose cough persists for more than 4 days exhibit significantly higher WBC levels than the healthy control while there is no statistical difference in WBC levels between the 1-3-day cough group and the healthy control group. Patients with an 8-15-day duration of cough have significantly elevated NLR levels compared to the healthy control group, with no statistical differences observed among other groups. The PLR levels in IgM-positive children are significantly higher than those in the healthy control group. Furthermore, children with a cough duration of 8-15 days had significantly higher PLR levels compared to those with 1-3-day duration of cough.

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Association Between IgM Antibody Concentration, WBC, NLR, PLR and Duration of Fever

The concentration of IgM antibodies in children with fever is significantly higher than that in the healthy control group (Table 3). Specifically, children with fever exceeding 3 days have significantly higher IgM antibody concentrations compared to those with no fever or with fever lasting 1-3 days. The WBC levels in the 1-3-day fever group are significantly higher than in both the healthy control group and the group with fever lasting more than 3 days. Children without fever have significantly higher WBC levels than the healthy control. The NLR levels in children with fever are higher than those in the healthy control group and the non-fever group; however, no statistical difference is observed between the healthy control group and the non-fever group. The PLR levels in IgM-positive children are significantly higher than those in the healthy control group. Additionally, children with fever lasting more than 3 days have significantly higher PLR levels compared to the non-fever group and the 1-3-day fever group.

Table 2. MP-IgM, WBC, NLR and PLR in children with different cough durations

Group	Case (n)	IgM (COI)	WBC (10^9/L)	NLR (%)	PLR (%)
1-3 days	22	2.46 ± 1.62^{ab}	7.38 ± 3.35^{b}	1.68 ± 1.56	98.66 ± 44.88^{ab}
4-7 days	59	2.8 ± 2.28^{ab}	8.39 ± 4.17^{a}	1.81 ± 1.79	113.09 ± 78.50^{a}
8-15 days	40	4.19 ± 3.47^{a}	9.24 ± 2.63^{a}	2.25 ± 2.35^{a}	141.31 ± 94.08^{a}
>15 days	28	2.77 ± 2.39^{ab}	8.29 ± 2.93^{a}	1.68 ± 2.33	105.46 ± 53.65^{ab}
Control	20	0.48 ± 1.07^{b}	6.47 ± 1.06^{b}	0.89 ± 0.32^{b}	46.84 ± 11.84^{b}
F value		7.74	2.27	1.72	6.10
P value		0.00	0.03	0.15	0.00

Note: $^{a}P < 0.05$ compared with control group; $^{b}P < 0.05$ compared with 8-15-day group.

Table 3. MP-IgM, WBC, NLR and PLR in different fever duration groups

Group	Case (n)	IgM (COI)	WBC (10^9/L)	NLR (%)	PLR (%)
Non-fever	61	2.82 ± 2.52^{ab}	8.52 ± 2.34^{a}	1.38 ± 1.00^{bd}	101.43 ± 50.53^{ac}
1-3 days	38	2.32 ± 1.92^{ab}	9.46 ± 4.10^{ab}	2.23 ± 2.17^{ac}	121.00 ± 76.70^{a}
>3 days	50	4.15 ± 2.98^{a}	7.59 ± 3.96^{d}	2.23 ± 2.36^{ac}	133.28 ± 96.77^{ac}
Control	20	0.48 ± 1.07^{b}	6.47 ± 1.06^{cd}	0.89 ± 0.32^{bd}	46.84 ± 11.84^{bcd}
F value		11.73	4.55	4.07	7.65
P value		0.00	0.04	0.08	0.00

Note: $^{a}P < 0.05$ compared with control group; $^{b}P < 0.05$ compared with >3-day group; $^{c}P < 0.05$ compared with non-fever group; $^{d}P < 0.05$ compared with the 1-3-day group.

DISCUSSION

MP represents a significant etiological agent for acute respiratory infection in children, characterised by its high contagion and prevalence as an epidemic pathogen among children.

Transmission of MP predominantly occurs through respiratory droplets and direct contact with an incubation period typically ranging 1-3 weeks. Patients remain infectious during both the incubation period and for several weeks after the amelioration of clinical symptoms [4-6]. Presently, clinical diagnosis of MP infection predominantly relies on clinical symptoms, epidemiological features and laboratory test results. Among the available laboratory methods, serological detection of MP-IgM is widely used in clinical practice [7].

Besides serological testing, quantitative MP-IgM testing is widely used in clinical outpatient settings for rapid analysis of both peripheral and venous blood because it is convenient, rapid, accurate and quantitative. It has demonstrated considerable applicative value in the early diagnosis of MP infection in children. Typically, MP-IgM appears 4-7 days after infection [8], yet differences in the incubation period and the onset of clinical symptoms such as cough and fever across children present challenges for clinical and laboratory departments in determining the optimal timing for conducting serum MP-IgM antibody testing.

Statistically, among the 149 MP-IgM positive pediatric cases studied, the earliest onset of cough symptoms was observed at one day after infection and fever at 0.5 day after infection, suggesting variability in the incubation period or antibody production capability in children. The latest occurrence of IgM positivity is marked by persistent coughing for up to 90 days and intermittent fever up to 30 days. The median duration of cough amongst IgM positive patients is 7 days, with 54.4% of MP-infected children testing antibody-positive within 7 days of cough symptom onset. Fever is observed in 59.1% of MP-IgM positive children, yet the incidence of fever declined in those with coughing of more than 7 days. Thus, in suspected MP infection with cough or fever symptoms, or cough persisting beyond 7 days without fever, MP-IgM antibody testing is recommended for rapid diagnosis.

The pathogenic mechanisms of MP include direct damage, indirect damage and immune damage, with potential inflammatory harm beyond the respiratory tract, affecting multiple organs and systems. Notably children with cough lasting 8-15 days have significantly higher IgM levels than those with cough lasting 1-7 days or more than 15 days (Table 2). As shown in Figure 1, 14 of the 40 patients in this group have fever lasting more than 3 days, which may partly explain the higher IgM levels observed. These findings are consistent with previous evidence that single serologic IgM testing may have limited sensitivity in the early stage of MP infection, whereas IgM detection becomes more informative as the disease course progresses [9].

It is worth noting that some children may exhibit low IgM titers or false-negative results in the early stage of infection, particularly those with immature immune systems or reinfection [10]. In such cases if clinical suspicion remains high despite negative IgM results, repeated testing after 3-5 days or combined detection with MP-PCR may improve diagnostic accuracy and reduce missed diagnoses [11].

Children with cough symptom exceeding 4 days exhibit significantly higher WBC levels compared to the control group, which is likely linked to MP-induced immune stress response. The NLR, a novel hematological marker, reflects the dynamic immune interplay between innate

neutrophil and adaptive lymphocyte response, displaying substantial sensitivity and prognostic value for systemic infections, sepsis and bacteremia diagnosis and stratification [12, 13]. Some children with MP pneumonia may develop microthrombosis and pleural effusion, leading to elevated platelet counts. Thus, IgM-positive children may show raised PLR, indicating the body's thrombotic and pre-thrombotic states [14, 15]. This study reveals that compared to the healthy control group, children coughing for 8-15 days and those with positive IgM exhibit significantly increased NLR and PLR levels respectively.

The timing of IgM positivity correlates with clinical symptoms and disease course, providing early indicators for disease prognosis and progression [16, 17]. This single-centre retrospective analysis through the examination of cough duration, febrile condition and persistence in the context of IgM, WBC, NLR and PLR data, offers empirical support for determining the optimal timing for conducting IgM testing in a clinical setting. In suspected MP infection cases with cough, fever or prolonged cough without fever for over 7 days, IgM antibody and complete blood count tests are recommended. A combined analysis of MP-IgM, WBC, NLR and PLR data can facilitate a rapid diagnosis of MP infection.

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