



# The diagnostic value of serological tests and real-time polymerase chain reaction in children with acute *Mycoplasma pneumoniae* infection

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**Background:** This study set out to evaluate the clinical significance and diagnostic effectiveness of serological tests and real-time polymerase chain reactions (RT-PCR) in children of different age groups and disease durations infected with *Mycoplasma pneumoniae* (MP).

**Methods:** Pediatric patients with lower respiratory tract infection (LRTI) confirmed by polymerase chain reaction (PCR) were enrolled and subjected to bronchoalveolar lavage fluid PCR (BALF-PCR) for MP infection. The diagnostic values of the serum immunoglobulin M (IgM) test, paired sera immunoglobulin G (IgG) test, RT PCR applied to nasopharyngeal aspirates (NPA-PCR), and combined IgM and NPA-PCR test were evaluated.

**Results:** When BALF PCR was used as the gold standard, the MP positivity rate of combined IgM and NPA PCR was 78.85% in children aged 3–5 years. The positivity rates of IgM, NPA PCR, and combined IgM and NPA PCR in children older than 5 years were 71.21%, 72.72%, and 84.85%, respectively. The detection rate of combined IgM and NPA PCR was consistent with BALF PCR (Kappa =0.727). The MP positivity rates of combined IgM and NPA PCR at 1–2 weeks was as high as 91.11%, and was consistent with the BALF PCR (Kappa =0.756). Moreover, the positivity rates of IgM or NPA PCR at 2–3 weeks were 63.16%, and were consistent with each other (Kappa =0.771).

**Conclusions:** Combined IgM and NPA PCR is the optimal test to confirm MP infection among children aged 3–5 years in cases with a disease duration of less than 2 weeks, and either NPA PCR or IgM is recommended for children older than 5 years with a disease duration of 2–3 weeks.

**Keywords:** *Mycoplasma pneumoniae* pneumonia (MPP); diagnosis; children; age; disease duration

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## Introduction

*Mycoplasma pneumoniae* (MP) is a prominent cause of infections of the upper and lower respiratory tract in children and has a particular association with community-acquired pneumonia (CAP) (1). In recent years, MP has been attributed with up to 40% of CAP cases in children (2-4). Based on its clinical presentation, *Mycoplasma pneumoniae* pneumonia (MPP) is often difficult to distinguish from pneumonia originating from other pathogens (5), and the problem has been exacerbated by the indiscriminate use of macrolides, which has resulted in the spread of drug-resistant strains of MP (6). Therefore, the prompt clinical treatment of MP infection hinges on rapid and reliable laboratory diagnosis.

Polymerase chain reaction (PCR) is a widely accepted method of diagnosis because of its speed and high sensitivity and specificity (7). Serological studies are more sensitive than culture for the detection of acute infections and have sensitivity comparable to PCR; however, they are dependent on antibodies, which require sufficient time to develop after infection and the patient to have a functional immune system (8). The immune system functions differently in children of different ages, which can lead to variations in innate and adaptive immune response and affect the expression of antibodies (9). Moreover, in some studies, the sensitivity of real-time polymerase chain reaction (RT-PCR) has been shown to decrease as the time between symptom onset and sample collection increases (9,10). Thus, an optimal detection method should be recommended for children of different ages and those with different disease durations, respectively. Therefore, we analyzed various early serological and molecular diagnostic tests to determine their clinical significance and usefulness in children of different ages infected with MP who had different disease durations. In investigating the optimal test, bronchoalveolar lavage fluid (BALF) PCR served as the gold standard (7,11,12).

## Methods

### Patients

The clinical information of pediatric patients with respiratory infection from January 2015 to October 2018 was collected and analyzed in this retrospective study. Patients were selected from a database which had been established for children with lower respiratory tract infections (LRTIs) in the Department of Respiratory Medicine, Children's Hospital, Soochow University.

We mainly enrolled pediatric patients under the age of 16 years who met the following criteria: (I) eligible for bronchoalveolar lavage procedures, with clinical manifestations of persistent chest radiographic infiltration and/or respiratory symptoms, such as wheezing, cough, increased dyspnea, unexplained hypoxemia; and (II) nasopharyngeal aspirates (NPA) and BALF samples were collected and analyzed by RT-PCR, and the serum titers of MP, immunoglobulin M (IgM) and immunoglobulin G (IgG), were checked in both the acute and convalescent phases. Patients with immunosuppression and those who were receiving immunosuppressive treatment were not included in the study.

A total of 296 children who met the criteria were enrolled, and 164 patients were confirmed mycoplasma infection by BALF PCR test. Detailed clinical history was recorded, and the following laboratory tests were conducted including blood routine, C-reactive-protein (CRP), lactate dehydrogenase (LDH), biochemical functions, humoral-immunity, and lymphocyte subsets. The study received approval from the Institutional Human Ethical Committee of Children's Hospital of Soochow University (No. 2020LW002), and written consent was obtained from the parent/guardian of each child participating in this study.

### Study method

#### Specimen collection

Venous blood samples were collected within 24 hours of admission and immediately sent to the laboratory. A second peripheral blood sample was collected from each patient before discharge. NPA samples were taken from the patients within 24 hours of admission via a sterile plastic catheter introduced through the nasal passage into the lower part of the pharynx. Bronchoscopy was performed under local anesthesia, following standard hospital procedures. Sterile normal saline was instilled into the most severely infected area or the right middle lobe with LRTI, and then sucked back into a sterile container with a negative pressure of 3.33–13.3 kPa. The 2–3 mL NPA specimens were immediately sent together with the first BALF aliquot to the laboratory for the detection of respiratory pathogens. All NPA and BAL procedures were performed by experienced staff in accordance with standardized clinical procedures.

#### Serology of MP

The ELISA kit (Shenzhen YHLO Biotech, Shenzhen,

China) was used to detect MP antibodies (IgM and IgG) in serum samples taken from the children in the acute phase (on admission) and convalescent phase (on discharge), in accordance with the manufacturer's instructions.

Acute MP infection was confirmed either by a single positive serum IgM titer (cut-off 1.1 S/CO) or a 1.5-fold increase in IgG titer (cut-off 24 RU/mL) in the convalescent serum sample (13).

### RT PCR for MP detection

A RT PCR procedure (DaAn Gene Co. Ltd., Guangzhou, China) was used to detect MP in real time (14) (approved by the State Food and Drug Administration of China). Briefly, both the samples of NPA and BALF were shaken for 30 s and centrifuged at 15,000  $\times$ g for 5 min. The sediment was collected and the DNA was extracted from a 400  $\mu$ L aliquot in line with the manufacturer's instructions. PCR amplification was performed using primers and probes purchased from DaAn Gene Co. Ltd., in a 7600 RT PCR system (Applied Biosystems, Foster City, CA, USA). The PCR conditions were as follows: 93 °C for 2 min; 10 cycles of 93 °C for 45 s and 55 °C for 60 s; 30 cycles of 93 °C for 30 s and 55 °C for 45 s. Quantitative curves were drawn for standard control samples at different concentrations. In the BALF samples, MP positive was indicated by MP DNA qualitative positive. To exclude MP colonization, in the NPA samples, MP-positive samples were defined as those with DNA concentrations  $\geq 1 \times 10^4$  copies/mL. In the combined IgM and NPA PCR test, MP-positive infection was indicated when either one or both of the tests were positive.

### Detection of co-infection with other respiratory pathogens

The BALF and NPA specimens were screened for bacteria using culture, and the quantitative specimen cultures were considered positive when the growth of  $\geq 10^5$  colony forming units per ml (cfu/mL) was observed. Respiratory viruses, such as influenza virus (IFV), respiratory syncytial virus (RSV), parainfluenza virus (PIV), and adenovirus (ADV) were detected by direct immunofluorescence assays (15), while human bocavirus (HBov) and rhino virus (HRV) were tested by reverse transcription PCR (16).

### Statistical analysis

Statistical analysis was carried out using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). Normal

distribution of measurement data was expressed as (mean  $\pm$  standard deviation), Non-normal distribution of measurement data was expressed as median (quartile spacing), and the two groups were compared using the Wilcoxon test. The chi-square test was applied for numerical data (Table 1). One-way analysis of variance (ANOVA) was used to calculate the differences in the positive rate between different groups (Figure 1, Tables 2,3). Correlation between the four different diagnostic methods was assessed by kappa analysis, and MP infection was diagnosed by BALF PCR. The validity of the different diagnostic methods, including specificity, sensitivity, and positive and negative predictive values were calculated with reference to the BALF PCR test results. Receiver operating characteristic (ROC) curves were plotted using SPSS 20.0 software, and the areas under the curves (AUCs) were calculated to assess significant differences between the diagnostic methods. Statistical significance was indicated when  $P < 0.05$  (Figure 2).

## Results

### Demographic and clinical features and the laboratory findings

The 296 patients enrolled in our study, of whom 182 (61.49%) were male and 114 (38.51%) were female, had a mean age of  $49.87 \pm 38.01$  months. The majority of the patients had a cough ( $n=292$ , 96.5%) or fever ( $n=220$ , 69.8%). The median duration of symptom onset was 7 days, and the median time between the paired sera collection for IgG quantification was 7.27 days. Overall, 164 patients tested positive for MP infection by BALF PCR. With a median age of  $58.71 \pm 37.33$  months, the patients who were MP positive were younger than those who tested MP negative ( $38.89 \pm 36.06$  months,  $P < 0.001$ ). On admission, most of the other symptoms were similar across both groups. The MP-positive groups had higher levels of LDH ( $P < 0.001$ ) and IgA ( $P = 0.001$ ), and a lower level of IgG ( $P < 0.001$ ). The MP-positive group positive also showed higher levels of CD3<sup>+</sup> cells ( $P < 0.001$ ) and CD3<sup>+</sup>CD4<sup>+</sup> cells ( $P = 0.016$ ), and a lower level of CD3<sup>-</sup>CD19<sup>+</sup> cells ( $P < 0.001$ ) (Table 1).

### Positive results of different laboratory tests in 164 children with MP infection confirmed by BALF PCR

Among the 164 children diagnosed with MP infection by

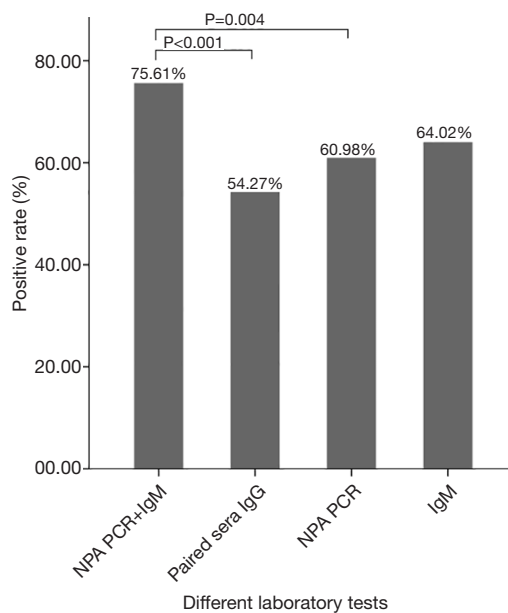
**Table 1** Demographic and clinical features and laboratory findings of patients

Parameters	All patients (n=296)	MP positive in BALF (n=164)	MP negative in BALF (n=132)	P value
Male/female	182:114	97:67	85:47	0.356
Age (months)	49.87±38.01	58.71±37.33	38.89±36.06	<0.001
Symptoms				
Cough [n (%)]	292 (96.5)	163 (99.39)	130 (98.48)	0.440
Wheezing [n (%)]	98 (31.1)	50 (30.49)	48 (36.36)	0.489
Fever [n (%)]	220 (69.8)	128 (78.05)	92 (69.70)	0.102
Tachypnea [n (%)]	29 (19.9)	8 (4.88)	21 (15.91)	0.002
Duration from onset (days)	7 [5–15]	8 [6–14]	7 [5–15]	0.352
Duration of hospitalization (days)	10.95±5.27	10.24±4.06	11.85±6.39	0.020
Duration of paired sera IgG taken apart (days)	7.27±2.84	7.22±2.79	7.34±2.93	0.878
Laboratory findings				
WBC (×10 <sup>9</sup> /L)	10.77±5.81	10.03±5.72	11.70±5.82	0.002
N (%)	59.05±19.1	59.24±18.71	58.58±19.75	0.90
PLT (×10 <sup>9</sup> /L)	325.33±114.67	313.99±110.25	339.43±118.86	0.069
CRP (mg/L)	8.81 (1.95–30.37)	9.5 (2.37–29.01)	7.31 (1.62–34.11)	7.87
LDH (U/L)	474.42±206.98	500.04±172.88	439.24±211.99	<0.001
Humoral immunity (g/L)*				
IgA	1.33 (0.61–5.19)	1.73 (0.76–7.24)	1.04 (0.36–1.88)	0.001
IgG	6.92 (1.50–9.77)	5.70 (1.23–8.98)	7.76 (4.55–10.61)	<0.001
IgM	1.11 (0.83–1.61)	1.22 (0.84–1.91)	1.02 (0.81–1.48)	0.29
Cell-mediated immunity (%)*				
CD3 <sup>+</sup>	63.62±10.47	65.95±9.49	60.48±10.93	<0.001
CD3 <sup>+</sup> CD4 <sup>+</sup>	33.67±8.68	34.58±8.58	32.45±8.71	0.016
CD3 <sup>+</sup> CD8 <sup>+</sup>	25.34±7.67	26.48±7.70	23.82±7.40	0.054
CD4 <sup>+</sup> /CD8 <sup>+</sup>	1.48±0.69	1.46±0.68	1.51±0.71	0.087
CD3 <sup>+</sup> CD19 <sup>+</sup>	23.30±10.52	21.07±9.33	26.29±11.30	0.001
CD3 <sup>+</sup> CD (16+56) <sup>+</sup>	11.63±7.89	11.37±6.88	11.98±9.09	0.387
CD19 <sup>+</sup> CD23 <sup>+</sup>	11.69±6.31	10.99±6.18	12.62±6.38	0.15

The data are presented as mean ± standard deviation, No./total No. (%), or median (quartile spacing). Comparison is conducted between the MP positive (n=164) and negative (n=132) groups tested by BALF-PCR. \*, the parameters were adjusted for age and analyzed by Kruskal-Wallis test. MP, Mycoplasma pneumoniae; BALF, bronchoalveolar lavage fluid; CRP, C-reactive-protein; LDH, lactate dehydrogenase; IgA, immunoglobulin A; IgM, immunoglobulin M; IgG, immunoglobulin G.

BALF PCR, 124 (75.61%) showed a positive result for combined IgM and NPA PCR, which was significantly higher than that for NPA PCR (n=100, 60.98%) and paired sera IgG (n=89, 54.27%), and 105 (64.02%) had a positive result for IgM (Figure 1).

With BALF PCR serving as the gold standard, the ROC curve was generated for each diagnostic test (Figure 2). The IgM and NPA PCR tests were shown to have the highest



**Figure 1** Positive results of different laboratory tests in 164 children with *Mycoplasma pneumoniae* infection proved by BALF PCR. NPAPCR + IgM = combined IgM and NPA PCR test.  $P < 0.05$  was considered statistically significant. BALF, bronchoalveolar lavage fluid; PCR, polymerase chain reaction; IgM, immunoglobulin M; NPA, nasopharyngeal aspirate.

diagnostic accuracy, and the area under the ROC curve for NPA PCR + IgM (0.775) was higher when compared with those for IgM (0.737) ( $P < 0.001$ ,  $\chi^2$  test) and paired sera IgG (0.503) ( $P < 0.001$ ,  $\chi^2$  test) alone. The area under the ROC curve for NPAPCR was 0.766. The sensitivity and specificity of combined IgM and NPA PCR were 82.12% and 72.41%, respectively, and the PPV and NPV of NPA PCR + IgM were 76.61% and 79.55%, respectively.

### Co-infection with MP

In the 164 children with MP infection confirmed by BALF PCR, MP was the only pathogen detected in 92 patients, while the remaining 72 (43.90%) had co-infection with other pathogens including respiratory viruses [37], HRV [12], HBoV [9], RSV [8], ADV [5], IFV-B [2], and IFV-A [1]. Bacterial infections were found in 19 patients, 2 were diagnosed with *Chlamydia pneumoniae* (CP), and 14 were positive for two or more additional pathogens.

### Comparison of MP positivity among different laboratory tests in children of different ages

The 164 children confirmed with MP infection by BALF PCR had a median age was  $58.71 \pm 37.3$  months and comprised 23 patients  $\leq 1$  year old, 23 patients aged 1–3 years old, 52 patients aged 3–5 years old, and 66 patients  $> 5$  years old. Positive results among the different age groups were compared using the same diagnostic test. The MP positive rate evaluated by IgM test in children  $< 1$  year old was 21.74%, which was lower than children aged 1–3, 3–5, and  $> 5$  years (all  $P < 0.0083$ ). The MP positive rate of NPA PCR test in children  $> 5$  years was 84.85%, which was higher than that of children  $< 1$  year old ( $P < 0.0083$ ). The

**Table 2** Positive results of different laboratory tests in 164 children of different ages infected with MP infection confirmed by BALF PCR

Parameters	$\leq 1$ -year-old (n=23)	1–3 years old (n=23)	3–5 years old (n=52)	$> 5$ years old (n=66)	P value
IgM [n (%)]	5 (21.74)	17 (73.91) <sup>a</sup>	36 (69.23) <sup>a</sup>	47 (71.21) <sup>a</sup>	$< 0.001$
Paired IgG [n (%)]	9 (39.13)	12 (52.17)	30 (57.69)	38 (57.58)	0.440
NPA PCR [n (%)]	7 (30.43)	15 (65.22)	30 (57.69)	48 (72.72) <sup>a</sup>	0.004
Combinated IgM and NPA PCR test [n (%)]	9 (39.13)	18 (78.26)	41 (78.85) <sup>a</sup>	56 (84.85) <sup>a,e</sup>	$< 0.001$
P value	0.537	0.245	0.064	0.007	–

Positive results of the same diagnostic test in different age groups were compared. <sup>a</sup>,  $P < 0.05$  compared with patients  $\leq 1$ -year-old. Positive results of different diagnostic tests in the same age group were compared. <sup>e</sup>,  $P < 0.05$  compared with the diagnostic tests of paired IgG. MP, *Mycoplasma pneumoniae*; BALF, bronchoalveolar lavage fluid; PCR, polymerase chain reaction; IgM, immunoglobulin M; IgG, immunoglobulin G; NPA, nasopharyngeal aspirate.

**Table 3** Comparison of MP positivity between different laboratory tests in patients of different ages

BALF PCR	IgM			Paired sera IgG			NPA PCR			Combined IgM and NPA PCR		
	(+)	(-)	Total	(+)	(-)	Total	(+)	(-)	Total	(+)	(-)	Total
In patients ≤1-year-old												
(+)	5	18	23	9	14	23	7	16	23	9	14	23
(-)	5	26	31	18	13	31	6	25	31	10	21	31
Total	10	44	54	27	27	54	13	41	54	19	35	54
Kappa	0.061			-0.185			0.117			0.07		
P value	0.6			0.169			0.346			0.601		
In patients 1–3 years old												
(+)	17	6	23	12	11	23	15	8	23	18	5	23
(-)	11	40	51	24	27	51	4	47	51	29	25	51
Total	28	46	74	36	38	74	19	55	74	31	30	74
Kappa	0.494			0.044			0.603			0.189		
P value	0			0.684			0			0.043		
In patients 3–5 years old												
(+)	36	16	52	30	22	52	30	22	52	41	11	52
(-)	3	22	25	17	8	25	2	23	25	3	22	25
Total	39	38	77	47	30	77	32	45	77	44	33	77
Kappa	0.504			-0.098			0.411			0.617		
P value	0			0.385			0			0		
In patients >5 years old												
(+)	47	19	66	38	28	66	48	18	66	56	10	66
(-)	1	24	25	14	11	25	0	25	25	1	24	25
Total	48	43	91	52	39	91	48	43	91	57	34	91
Kappa	0.549			0.06			0.594			0.727		
P value	0			0.542			0			0		

MP, Mycoplasma pneumoniae; IgM, immunoglobulin M; IgG, immunoglobulin G; NPA, nasopharyngeal aspirate; BALF, bronchoalveolar lavage fluid; PCR, polymerase chain reaction.

MP positive rate evaluated by the combined IgM and NPA PCR test in children aged 3–5 years and those >5 years was 78.85% and 84.85%, respectively, which was higher than the results of children <1 year ( $P<0.0083$ ). Comparisons were also made between the positive results of the different diagnostic tests on patients of the same age. The MP positive rate of the combined IgM and NPA PCR test in children >5 years old was 84.85%, which was higher than that of the paired sera IgG test ( $P<0.0083$ ) (Table 2).

When BALF PCR was used as the gold standard, the

MP detection rate of the NPA PCR test in children aged 1–3 years was consistent with that of the BALF PCR test (Kappa =0.603). The MP detection rate of the combined IgM and NPA PCR test in children aged 3–5 years old was consistent with that of BALF PCR test (Kappa =0.617). In children >5 years, the MP detection rate of the combined IgM and NPA PCR test was consistent with that of BALF PCR test (Kappa =0.727), and the detection rates of the independent NPA PCR and IgM tests were both moderately consistent with that of the BALF PCR test (Kappa =0.549,

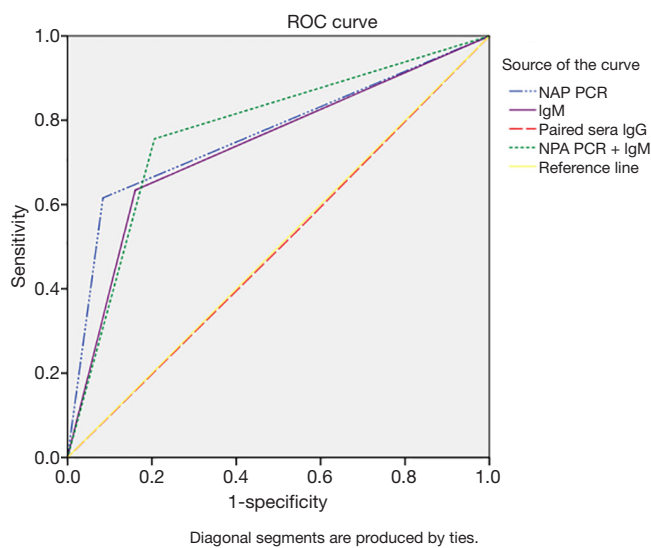
0.594). Moreover, there was a correlation between the detection rates of the NPA PCR and IgM tests (Kappa =0.824) (Table 3).

### Comparison of MP positivity among different laboratory tests in children with different disease durations

Among the 164 children diagnosed with MP infection by BALF PCR, the median duration of symptom onset was 7 days. Regarding disease duration, 80, 45, 19, and 20 patients had a disease duration <1, 1–2, 2–3 weeks, and

>3 weeks, respectively. Comparisons were made between the different diagnostic tests for children with different disease durations. The positivity rates evaluated by IgM test in children with a disease duration of 1–2 weeks was 82.22%, which was higher than that of children with a duration <1 week and >3 weeks (all  $P < 0.0083$ ). The MP positive rates of the paired sera IgG test in children with a disease duration <1 week was 70%, which was higher than that of children with a duration of 2–3 weeks or >3 weeks (all  $P < 0.0083$ ). The MP positive rates of the combined IgM and NPA PCR test in children with a disease duration <1 week or 1–2 weeks was 70% and 91.11%, respectively, which was higher than that of >3 weeks (all  $P < 0.0083$ ). Comparisons were made between the positive results of the different diagnostic tests in patients with the same duration from onset. The MP positive rates of the NPA MP + IgM test in children with a disease duration of 1–2 weeks was 91.11%, which was higher than that of the paired sera IgG test and NPA PCR test (all  $P < 0.0083$ ). The MP positive rates of the combined IgM and NPA PCR test in children with a duration of 2–3 weeks was 68.42%, which was higher than that of the paired sera IgG test (all  $P < 0.0083$ ) (Table 4).

When BALF PCR was used as the gold standard for diagnosis of children with a disease duration <1 week, the MP detection rate of the IgM test was moderately consistent with that of the BALF PCR test (Kappa =0.505), and the detection rates of the NPA PCR test and IgM were not consistent with each other (Kappa =0.438). The detection rate of the combined IgM and NPA PCR test in children with a disease duration of 1–2 weeks was correlated with that of the BALF PCR test (Kappa =0.756). In children with a disease duration of 2–3 weeks, none of the four diagnostic tests were



**Figure 2** The receiver operating curves (ROC) for different laboratory tests. NPA PCR + IgM = combined IgM and NPA PCR test.

**Table 4** Positive results of different laboratory tests in 164 children with different disease durations infected with MP infection confirmed by BALF PCR

Parameters	≤1 week (n=80)	1–2 weeks (n=45)	2–3 weeks (n=19)	>3 weeks (n=20)	P value
IgM [n (%)]	46 (57.50) <sup>b</sup>	37 (82.22)	12 (63.16)	10 (50.00) <sup>b</sup>	0.022
Paired sera IgG [n (%)]	56 (70.00)	22 (48.89)	5 (26.32) <sup>a</sup>	6 (30.00) <sup>a</sup>	0.000
NPA PCR [n (%)]	50 (62.50)	30 (66.67)	12 (63.16)	8 (40.00)	0.219
Combined IgM and NPA PCR [n (%)]	61 (76.25)	41 (91.11) <sup>f,g</sup>	13 (68.42) <sup>f</sup>	9 (45.00) <sup>a,b</sup>	0.001
P value	0.062	0.000	0.033	0.614	—

Positive results of the same diagnostic test on patients with different disease durations were compared. <sup>a</sup>,  $P < 0.05$  compared with the patients with disease duration ≤1 week; <sup>b</sup>,  $P < 0.05$  compared with the patients with disease duration 1–2 weeks. Positive results of different diagnostic tests on patients with the same disease duration were compared. <sup>f</sup>,  $P < 0.05$  compared with the paired sera IgG diagnostic test; <sup>g</sup>,  $P < 0.05$  compared with the NPA PCR diagnostic test. MP, Mycoplasma pneumoniae; BALF, bronchoalveolar lavage fluid; PCR, polymerase chain reaction; IgM, immunoglobulin M; IgG, immunoglobulin G; NPA, nasopharyngeal aspirate.

**Table 5** Comparison of MP positivity among different laboratory tests in patients with different disease durations

BALF PCR	IgM			Paired sera IgG			NPA PCR			Combined IgM and NPA PCR		
	(+)	(-)	Total	(+)	(-)	Total	(+)	(-)	Total	(+)	(-)	Total
In patients with disease duration $\leq 1$ week												
(+)	46	34	80	56	24	80	50	30	80	61	19	80
(-)	2	57	59	29	30	59	15	44	59	36	23	59
Total	48	91	139	85	54	139	65	74	139	97	42	139
Kappa	0.505			0.211			0.359			0.158		
P value	0			0.013			0			0.053		
In patients with disease duration of 1–2 weeks												
(+)	37	8	45	22	23	45	30	15	45	41	4	45
(-)	2	14	16	4	12	16	1	15	16	2	14	16
Total	39	22	61	26	35	61	31	30	61	43	18	61
Kappa	0.622			0.173			0.471			0.756		
P value	0			0.097			0			0		
In patients with disease duration of 2–3 weeks												
(+)	12	7	19	5	14	19	12	7	19	13	6	19
(-)	6	20	26	10	16	26	7	19	26	9	17	26
Total	18	27	45	15	30	45	19	26	45	22	23	45
Kappa	0.404			-0.125			0.362			0.331		
P value	0.007			0.393			0.015			0.025		
In patients with the disease duration $>3$ weeks												
(+)	10	10	20	6	14	20	8	12	20	12	8	20
(-)	6	25	31	13	18	31	11	20	31	11	20	31
Total	16	35	51	19	32	51	19	32	51	23	28	51
Kappa	0.318			-0.120			0.046			0.239		
P value	0.021			0.389			0.745			0.086		

MP, Mycoplasma pneumoniae; IgM, immunoglobulin M; IgG, immunoglobulin G; NPA, nasopharyngeal aspirate; BALF, bronchoalveolar lavage fluid; PCR, polymerase chain reaction.

consistent with that of BALF PCR test, while the detection rates of NPA PCR test and IgM were well correlated with each other (Kappa =0.771) (Table 5).

## Discussion

Early diagnosis of MP infection in children remains clinically challenging due to its nonspecific clinical manifestations as well as a lack of both accurate and quick laboratory tests. Diagnosis of MP by culture is a

traditional gold standard test, but it is time-consuming and cumbersome. Recent studies have suggested that applying PCR to BALF could serve as a new gold standard test for diagnosing MP infection because of its high sensitivity and specificity, and can minimize cases of colonization (7,11,12). In our study, BALF PCR test was used as the gold standard to compare different diagnostic methods for early detection of MP infection in children.

In our study, 164 patients tested positive for MP infection by BALF PCR, with a positive rate of 55.41%, which is



higher than that reported in previous studies (5,17). Many of the children enrolled in this study were already suffering with lobar pneumonia, showed persistent chest radiographic infiltration, and were eligible for bronchoalveolar lavage procedures. Moreover, MP was established in our previous studies as the major pathogen of lobar pneumonia, accounting for 88% of all pathogens (date not published).

The serological assay, which relies on the pathogen-induced production of antibodies, is often used to diagnose MP infection. Various assays, such as direct and indirect hemagglutination assays that use IgM capture, immunofluorescence antibody assays, particle agglutination antibody assays, and enzyme immunoassays (EIAs) are widely available. IgM is considered to be an early indicator of MP infection, as it appears in the first week of the disease and reaches peak titers in the third week. After declining to low basal titer levels it may be present for up to several years (8); therefore, IgM detection is more reliable for diagnosing children than elderly people (18). Two recent studies have reported the positivity rate of MP-specific IgM for pediatric patients as 48.2% and 63.6%, respectively (19,20). In our study, the positivity of IgM test was 64.02%, but its value was limited for diagnosing MP infection in children under 1 year of age, for whom the positivity rate was only 21.74% compared with 71.21% for children over 5 years of age. Since the antibody response to IgM is not often induced in very young children (21). The children with a disease onset of 1–2 weeks showed the highest positivity rate (82.22%), and a detection rate consistent with BALF PCR. Previous studies by Lee *et al.* showed that 32.5% of patients with MP did not have a positivity IgM titer during the first several days of symptoms, and IgM only became positive after 2 weeks of follow up (19). Our results were consistent with these earlier reports.

As is commonly understood, a positive conversion or a significant increase in IgG antibody titer in paired sera may take at least 2 weeks, and can indicate an acute MP infection. However, taking two consecutive blood samples 2 weeks apart is too late to assist with guiding treatment decisions, and it is sometimes difficult to perform clinically in children (17). In our study, the median time gap of the collection of paired sera was 7.27 days, which explains the decrease in positivity rates (54.24%) of paired sera IgG. A recent study also found that the early IgG samples in patients taken within 7 days of symptoms showed a poor positivity rate (10.7%) (20). Interestingly, the positivity rate of paired sera IgG in pediatric patients taken within 1 week of illness onset was as high as 70.00%, which was higher than those with a gap

of longer than 2 weeks. This may be because IgG typically rises after 2 weeks of illness, and we collected the first sera within 1 week of illness, while the second sera was collected after 2 weeks having undergone a positive conversion or significant increase in IgG. However, the detection rate was not consistent with that of BALF PCR. These findings indicate that paired sera collected with less than 2 weeks of disease duration may be of limited value for diagnosing MP infection. Instead, IgM may be a reliable specific indicator of infection in pediatric patients older than 5 years and those with the disease duration of 1–2 weeks.

PCR is accepted as a rapid diagnostic test, and because of its high sensitivity, speed, and specificity, some studies have suggested that PCR tests for MP could replace serology and culture as the “new gold standard” (22). However, PCR is not readily available in many laboratories and may not be suitable for diagnosis in some cases, such as those affected by colonization or prolonged shedding from previous episodes of infection. Spuesens *et al.* found that by using the PCR method, MP DNA could be detected in 16.2% healthy pediatrics who did not display any respiratory symptoms, and that the MP in the upper respiratory tract could persist for up to 4 months (23). Therefore, it is necessary for physicians to differentiate between a true MP acute infection and a carrier and colonized situation.

An early retrospective study suggested that a high bacterial load was indicative of an MP infection (24), and a threshold of  $10^4$  genomic DNA copies per mL can be applied to distinguish clinical infection from carriage (11). To minimize bias induced by cases of colonization in the study, we only enrolled patients showing clinical signs and symptoms compatible with mycoplasma infection, along with MP PCR detection in both NPA and BALF, and defined DNA concentrations  $\geq 1 \times 10^4$  copies/mL as positive MP infection in the NPA samples. The positivity rate of NPA PCR in our study was 64.02%, but in pediatric patients younger than 1 year it was only 30.43%. In those aged between 1–3 years and older than 5 years of age, the positivity rates were 65.22% and 72.72%, respectively, and the detection rates were both consistent with BALF PCR. This showed that the NPA PCR assay may be of good diagnostic value for pediatric patients aged between 1–3 years old and younger than 5 years old.

A previously published research report stated that the detection of IgM antibodies in combination with PCR allowed for a precise and reliable MP diagnosis during the acute phase of the disease (17). In our study, patients were defined as infected with MP if they tested positive for IgM

antibodies and/or PCR, and the combined IgM and NPA PCR test showed the highest positivity rates (75.61%). With BALF PCR as the gold standard, the area under the ROC curves for the combined IgM and NPA PCR test was significantly higher. However, our study also found that, even with combined IgM and NPA PCR test, the positivity rate for patients younger than 1 year was only 39.13%, similar to those of NPA PCR, IgM, and paired sera IgG alone. The positivity rates of combined IgM and NPA PCR in pediatric patients aged between 1–3 years old and older than 5 years were 78.85% and 84.85%, respectively, and the detection rates were both consistent with BALF PCR. Combined IgM and NPA PCR was also proved to be of great value for pediatric patients with a duration from onset of 1–2 weeks, which showed a positivity rate as high as 91.11% and detection rates consistent with BALF PCR.

This study has some limitations. Firstly, using bronchoalveolar lavage fluid PCR assay as a gold standard to compare different diagnostic methods is not irrefutable. PCR assays may have false-negative results due to inhibitors in samples or poor technique. Secondly, in our study the median time between the paired sera IgG being taken was only 7.27 days. Lastly, our sample size was relatively small, and further research with larger sample sizes is needed to support our findings.

## Conclusions

In conclusion, when evaluating pediatric patients with acute MP infection, our results suggested that, together with compatible clinical history, the combined IgM and NPA PCR is an optimal test for confirming MP infection for pediatric patients aged between 3–5 years and those with a disease onset of less than 2 weeks. NPA PCR or IgM each have prime diagnostic value for pediatric patients over 5 years of age and those with the disease onset of 2–3 weeks. NPA MP-PCR is the most useful test for diagnosing patients aged between 1–3 years. For pediatric patients under 1 year, and those with a disease onset more than 3 weeks, none of the four testing methods in this study are valid and a new diagnostic method should be investigated.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Institutional Human Ethical Committee of Children's Hospital of Soochow University (No. 2020LW002). Written consent was obtained from the guardians of each child who participated in this study.

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