Journal of Microbiology, Immunology and Infection (2014) **47**, 137–144

Available online at www.sciencedirect.com

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ORIGINAL ARTICLE

# Comparison of real-time polymerase chain reaction and serological tests for the confirmation of *Mycoplasma pneumoniae* infection in children with clinical diagnosis of atypical pneumonia

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Received 30 April 2012; received in revised form 30 June 2012; accepted 31 August 2012
Available online 29 May 2013

**KEYWORDS**
Immunoglobulin M;
*Mycoplasma pneumoniae*;
Polymerase chain reaction

*Background: Mycoplasma pneumoniae* is a common pathogen of respiratory tract infection in children, and its correct and rapid diagnosis is a clinical challenge. Real-time polymerase chain reaction (RT-PCR) has been used frequently for the detection of this pathogen.
*Materials and methods:* Medical records from all children with a clinical diagnosis of mycoplasma pneumonia and whose respiratory samples were tested for *M. pneumoniae* (using RT-PCR) during 2011 were reviewed retrospectively. We compared the sensitivity and specificity of serological assays versus those of RT-PCR for diagnosis of *M. pneumoniae* infections. We also reviewed retrospectively clinical characteristics, and laboratory and imaging findings of children with laboratory evidence of *M. pneumoniae* infection.
*Results:* In 2011, 290 children were diagnosed to have mycoplasma pneumonia clinically and had their respiratory samples tested for *M. pneumoniae* by RT-PCR. Fifty-four children (19%) had a positive result. Meanwhile, 63% (182/290) of these children also underwent serological tests, out of whom 44 (24%) were found to be positive for immunoglobulin M (IgM). Using PCR as a gold standard, *M. pneumoniae* IgM assay was found to show a sensitivity of 62.2% and a specificity of 85.5%. Positive and negative predictive values of IgM were 52.3% and 89.9%, respectively. In *M. pneumoniae* IgM-positive children, a negative PCR result was associated with more coinfection by other pathogens and longer duration of prehospitalization fever. Bacterial loads of *M. pneumoniae* were not correlated with clinical outcomes.

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http://dx.doi.org/10.1016/j.jmii.2013.03.015

*Conclusion:* The majority of clinically diagnosed mycoplasma pneumonia was unconfirmed. *Mycoplasma pneumoniae* IgM has poor sensitivity and a positive predictive value. Interpretation of *Mycoplasma pneumoniae* IgM should be done with caution.


## Introduction

*Mycoplasma pneumoniae* is a common cause of respiratory tract infection in all age groups, including children and adolescents. It has been reported to be the cause of up to 10—40% of all community-acquired pneumonia.[1—3] Awareness of *Mycoplasma* is nowadays widespread among pediatricians and even among parents. However, confirmation of *M. pneumoniae* infection is clinically challenging. Indiscriminate use of macrolides has led to an increased resistance to it in *M. pneumoniae*. Culture of this pathogen is difficult to perform and time consuming. Detection of antibody conversion or four-fold rise in antibody titers in two consecutive sera samples collected 2 weeks apart indicates an acute infection. However, this is also time consuming and offers a diagnosis only retrospectively. In addition, the enzyme-linked immunosorbent assay (ELISA) methods that are currently being used widely for the detection of *Mycoplasma* immunoglobulin G (IgG) are mostly semiquantitative and not useful in detecting the four-fold rise of antibody titers. The sensitivity and specificity of *M. pneumoniae* IgM remained elusive. It may require 1—2 weeks for IgM antibodies to develop. All these factors limited clinical use of serological assays in diagnosing *M. pneumoniae* infections. Although the real-time polymerase chain reaction (RT-PCR) technology has advantages, it also has its limitations, such as being expensive.[4,5] Currently, diagnosis of *M. pneumoniae* infection mainly relies on serological tests. However, PCR-based assays are increasingly being adopted, because recent studies concluded that PCR is superior to serology for the diagnosis of *M. pneumoniae* infection during its early phase.[6,7]

In this study, we compared the results of *Mycoplasma* IgM and RT-PCR assays in a clinical setting. Because we did not have other reference standards for diagnosing *M. pneumoniae* infection, we evaluated the sensitivity and specificity of *Mycoplasma* IgM for diagnosing *M. pneumoniae* infection using PCR as a gold standard. Clinical and laboratory characteristics of patients with laboratory evidence, either serology or PCR, were analyzed. Comparisons were made between PCR-positive and PCR-negative patients among IgM-positive patients. In addition, the relation between *Mycoplasma* loads and severity of disease was also investigated.

## Materials and methods

This is a retrospective analysis of clinical information from pediatric patients (aged $\leq$18 years), diagnosed to have mycoplasma pneumonia, in a children hospital. An in-house RT-PCR system for *M. pneumoniae* was established. Respiratory samples (throat swab, sputum, or pleural effusion) of children with *Mycoplasma* infection-compatible clinical symptoms (fever and cough with minimal or no respiratory distress), physical findings (crackles or wheezing on auscultation), and radiological findings (patchy or linear infiltrates on chest X-ray) were checked for *M. pneumoniae* DNA by RT-PCR on a clinical service basis. Most of these children were also checked for *M. pneumoniae* IgG/IgM, blood culture, virus isolation, and urine pneumococcal antigen when feasible.

Sensitivity, specificity, and positive and negative predictive values were calculated for IgM using PCR result as a diagnostic standard.[7] Clinical features, laboratory findings, and chest radiographic findings for patients with positive *M. pneumoniae* IgM or PCR were retrospectively reviewed. The chest radiographic findings at admission were divided into two patterns: bronchopneumonia or lobar pneumonia. Bronchopneumonia was defined as increased nodular densities along bronchial trees or/and an interstitial pattern on unilateral or bilateral lung fields. Lobar pneumonia was defined as a distinctive segmental or lobar consolidation. Comparisons were made between PCR-positive and PCR-negative patients.

### RT-PCR for *M. pneumoniae*

Samples for RT-PCR analysis were placed in tubes containing phosphate-buffered saline and were vortexed. DNA was extracted from 200 μL of each sample using the Roche MagNA Pure LC total nucleic acid isolation kit (MagNA Pure LC 2.0; Roche, Basel, Switzerland) and finally eluted with 100 μL elution buffer. A quantitative RT-PCR method was used to measure the load of *M. pneumoniae*. Primers MP-Forward (CCAACCAAACAACAACgTTCA) and MP-Reverse (TAACGGCAACACGTAATCAGGTC) along with the probe MP-probe (6FAM-ACCTTGACTGGAGGCCGTTA-BHQ1) were used to identify the Cytadhesin P1 (130 bp) target gene.[8] RT-PCR reaction was programmed by DyNAmo Flash Probe qPCR kit (New England Biolabs, Vantaa, Finland) and LightCycler 1.5 instrument (Roche).

### *M. pneumoniae* serology

IgM or IgG antibodies were measured using the SeroMP IgM or IgG kit (Savyon Diagnostics Ltd, Ashdod, Israel), which is a semiquantitative ELISA, for the determination of antibodies specific to *M. pneumoniae*. The antigen used in the SeroMP kit is the P1 membrane protein, which is a major immunogen of *M. pneumoniae*. Based on the manufacturer's suggestion, a negative IgM or IgG reaction was defined as $<$10 BU/mL, an equivocal IgM or IgG reaction as

10–20 BU/mL, and a positive IgM or IgG reaction as ≥20 BU/mL.

### Statistical analysis

We compared the data of two separate groups using the Chi-square test or Fisher's exact test for categorical variables and the Mann–Whitney *U* test for continuous variables. A *p* value of <0.05 was considered significant. All the statistical operations were two tailed and performed with SPSS version 19 (SPSS Inc., Chicago, IL, USA).

## Results

In 2011, in 290 pediatric patients with a clinical diagnosis of mycoplasma pneumonia, RT-PCR assay for *M. pneumoniae* was performed on the throat swab ($n = 278$), sputum ($n = 9$), or pleural effusion ($n = 3$). Fifty-four (19%) patients had positive results (50 throat swabs, 2 sputum, and 2 pleural effusion). In all PCR-positive and hospitalized patients ($n = 41$), the PCR was performed 7.8 ± 3.3 days after disease onset. In total, 244 patients were hospitalized. *M. pneumoniae* serology assays were carried out for 182 (74.6%) of these patients. Among them, 44 (24.2%) had a positive IgM. In hospitalized patients who received both PCR and serology assays, 31.9% (58/182) were positive for either IgM or PCR. Twenty-three patients were positive for both *M. pneumoniae* IgM and PCR at the same time (Fig. 1). *M. pneumoniae* bacterial loads were also determined by RT-PCR. Among all the PCR-positive patients, the median load of *M. pneumoniae* was $5.4 \times 10^5$ copies/mL, ranging from $1.2 \times 10^3$/mL to $3.3 \times 10^8$/mL. Patients with a clinical diagnosis of mycoplasma pneumonia or positive *M. pneumoniae* PCR ($n = 54$) were encountered all year round. PCR-positive case numbers in July and October seemed higher than those in other months of the year (Fig. 2). However, there was no clear seasonality variation.

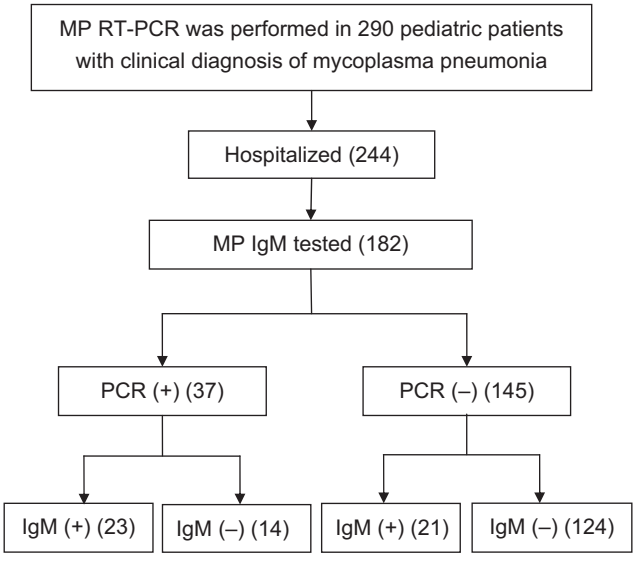


**Figure 1.** Flow chart of the study design and number of cases analysis. Data in parentheses denote case numbers. IgM = immunoglobulin M; MP = *M. pneumoniae*; RT-PCR = real-time polymerase chain reaction.

### Sensitivity, specificity, and positive and negative predictive values of *M. pneumoniae* IgM and RT-PCR

Among all the hospitalized patients with a clinical diagnosis of mycoplasma pneumonia, 182 received both *M. pneumoniae* PCR and IgM assays. The results are shown in Table 1. Because there was no reference standard available for the diagnosis of *Mycoplasma* infection, IgM assay for *M. pneumoniae* was evaluated using *M. pneumoniae* PCR as the "gold standard." Sensitivity and specificity of IgM were estimated to be 62.2% and 85.5%, respectively. Positive predictive value of IgM was 52.3% and negative predictive value was 89.9%. A receiver operating characteristic (ROC) curve of *M. pneumoniae* IgM was figured according to a different cutoff value (Fig. 3). The ROC curve showed that 25 BU/mL was the most adequate cutoff point of *M. pneumoniae* IgM. Under such a cutoff value, sensitivity remained the same (62.2%) and specificity increased a little to 90.3% (95% confidence interval: 0.65–0.86; area under curve (AUC) = 0.76).

By contrast, when IgM serology was used as a gold standard, sensitivity and specificity of RT-PCR became 52.3% and 89.9%, respectively. Positive predictive value of PCR was 62.2% and negative predictive value was 85.5%.

### Clinical and laboratory findings in patients with *M. pneumoniae* infections

Clinical features, laboratory, and chest radiographic findings of hospitalized patients who had laboratory evidence (either PCR or IgM) of *M. pneumoniae* infection ($n = 58$) were reviewed. Urine pneumococcus Ag was checked in 60% (35/58) of these patients, with 9% (3/35) showing positive results. About a quarter (15/58, 25.9%) of these patients had coinfections with adenovirus, pneumococcus, or others (Table 2). Patients with coinfections ($n = 15$) were excluded from further analysis. The mean age of the patients was 7.6 ± 3.8 years, and male-to-female ratio was 0.72:1. All patients had fever and cough. The total duration of fever was 8.3 ± 4.2 days (5.7 ± 3.5 days prior to admission and 2.5 ± 3.0 days after admission). About two-

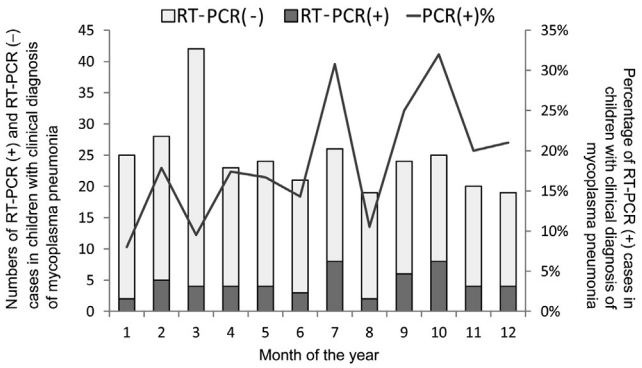


**Figure 2.** Number of patients with positive and negative *Mycoplasma pneumoniae* RT-PCR results according to months of the year. RT-PCR = real-time polymerase chain reaction.

**Table 1** Sensitivity, specificity, and positive and negative predictive values of *Mycoplasma pneumoniae* IgM using real-time PCR as a diagnostic standard

| | PCR (+) | PCR (−) | Total |
|---|---|---|---|
| IgM (+)[a] | 23 | 21 | 44 |
| IgM (−) | 14 | 124 | 138 |
| Total | 37 | 145 | 182 |
| Sensitivity | 23/37 = 62.2% | | |
| Specificity | 124/145 = 85.5% | | |
| PPV | 23/44 = 52.3% | | |
| NPV | 124/138 = 89.9% | | |

[a] An IgM titer value of 20 BU/mL was defined as positive.
IgM = immunoglobulin M; NPV = negative predictive value; PCR = polymerase chain reaction; PPV = positive predictive value.

thirds (27/43, 63%) of them had fever for over 7 days. On average, fever subsided 1.9 days after the first dose of azithromycin.

The majority of patients had abnormal breathing sounds, and nearly one-fourth of the patients had mild chest wall retractions. Lobar pneumonia and bronchopneumonia accounted for 98% of RT-PCR-positive patients. Chest X-ray revealed lobar pneumonia in two-thirds (27/43, 63%) of the patients. One-fourth (26%, 11/43) of them had pleural effusion, as confirmed by chest echo. Three children received thoracocentasis and two of them had positive *M. pneumoniae* RT-PCR result from pleural effusions. Skin rash was noted in three (7%) patients. Patients in whom antibody determination was performed 7 days or more after the onset of fever were more likely to be positive for IgM [17/21 (81%) vs. 6/16 (38%), respectively; $p = 0.03$].

Among these 37 hospitalized, *M. pneumoniae* PCR-positive patients, eight were treated with azithromycin prior to hospitalization. After hospitalization, five of these eight patients received a second course of azithromycin. By contrast, 25 of 29 patients who were not treated with macrolides prior to hospitalization received azithromycin treatment after hospitalization. Overall, 11% (4/37) of the patients did not receive any mycoplasma-specific treatment throughout their clinical courses. Fever subsided in 2.5 ± 4.7 days after the first postadmission dose of azithromycin. Patients who received macrolide treatment prior to hospitalization ($n = 8$) had a longer total duration of fever than those who did not ($n = 29$) (14.4 ± 7.5 vs. 7.8 ± 2.8 days; $p = 0.001$). Patients who were not treated with macrolides ($n = 4$) had an average fever duration of 8.3 ± 3.6 days, which is not significantly different from those treated with azithromycin (9.3 ± 5.1 days, $n = 35$; $p = 0.73$).

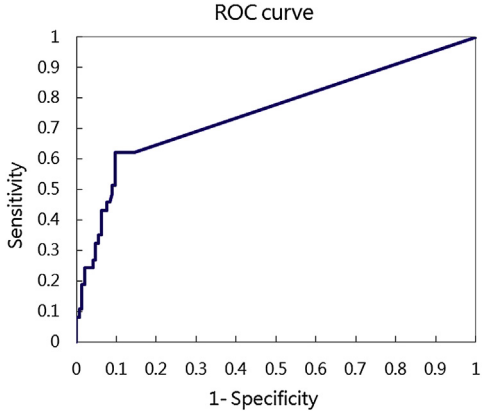


**Figure 3.** ROC curve of *Mycoplasma pneumoniae* IgM. Sensitivity and specificity were 62.2% and 90.3%, respectively (95% CI = 0.65—0.86; area under curve (AUC) = 0.76) at 25 BU/mL, the most adequate cutoff point of IgM. AUC = area under the curve; CI = confidence interval; IgM = immunoglobulin M; ROC = receiver operating characteristic.

Only two patients needed ventilator support due to impending respiratory failure. Both of them had lung consolidation and pleural effusion. One of them was coinfected with adenovirus and complicated with acute respiratory distress syndrome. However, no evidence of coinfection could be found in the other patient who was intubated. He was then extubated within 1 week. Almost all patients had good outcome and recovered without any complications. Only one patient had a sequela of neurological regression due to the complication of extracorporeal membrane oxygenation (ECMO) settings for a period of time.

Among patients with positive *M. pneumoniae* IgM, those with negative PCR results had more coinfections than those whose PCR results were positive (Table 2). When patients with coinfections ($n = 32$) were excluded, 20 (63%) patients were also found to be positive for *M. pneumoniae* PCR and the other 12 (37%) were PCR negative. Clinical and laboratory findings of these two groups were compared (Table 3). The duration of fever prior to hospitalization in PCR-positive group was 6.7 ± 3.2 days, which was significantly longer than that of PCR-negative group (4.7 ± 4.8 days; $p = 0.04$). The total duration of fever was not significantly different between the two groups.

Among the 43 hospitalized patients who were positive for *M. pneumoniae* PCR or IgM and had no detectable coinfections, 27 (63%) belonged to lobar pneumonia group, whereas 16 (37%) were classified as bronchopneumonia group. Comparisons of parameters such as disease severity, including duration of fever, duration of hospitalization, intensive care unit (ICU) stay, white blood cell (WBC) counts, *M. pneumoniae* PCR load, ventilator requirement, and clinical outcome between these two groups revealed no significant differences (Table 4). Furthermore, we also compared disease severity parameters, including fever duration, hospitalization, ICU stay, WBC counts, C-reactive protein, and treatment requirements for different *M. pneumoniae* PCR loads (e.g., $1 \times 10^4$/mL, $1 \times 10^5$/mL, $1 \times 10^6$/mL, and $1 \times 10^7$/mL). There was no obvious association between *M. pneumoniae* load and disease severity.

## Discussion

Our results showed that in pediatric patients who were diagnosed with mycoplasma pneumonia clinically, only a small proportion of them were confirmed by laboratory tests, either the detection of *M. pneumoniae* IgM by ELISA

**Table 2** Number of cases with respiratory pathogens other than *Mycoplasma pneumoniae*, according to *M. pneumoniae* PCR and IgM results[a]

| | PCR (+)/IgM (+) $n = 58$ | PCR (+)/IgM (+) $n = 23$ | PCR (−)/IgM (+) $n = 21$ | PCR (+)/IgM (−) $n = 14$ |
|---|---|---|---|---|
| Adenovirus | 8 | 2 | 6 | |
| Pneumococcus | 2 | | 1 | 1 |
| Adenovirus plus pneumococcus | 1 | | 1 | |
| Influenza B | 2 | | | 2 |
| Coxsackievirus B5 | 1 | 1 | | |
| Epstein—Barr virus | 1 | | 1 | |
| Total | 15 | 3 | 9 | 3 |

[a] Adenovirus, influenza B, and Coxsackievirus B5 were identified by virus culture of throat swabs. Epstein—Barr virus was diagnosed by serology. Pneumococcus was diagnosed by urine pneumococcal antigen.
IgM = immunoglobulin M; PCR = polymerase chain reaction.

**Table 3** Clinical and laboratory characteristics of various groups of hospitalized children with clinical diagnosis of mycoplasma pneumonia[a]

| | PCR (+) or IgM (+) $n = 43$ | PCR (+) and IgM (+) $n = 20$ | PCR (−) and IgM (+) $n = 12$ | PCR (+) and IgM (−) $n = 11$ | $p$[b] |
|---|---|---|---|---|---|
| Age (y) | 7.6 ± 3.8 | 6.9 ± 3.7 | 7.0 ± 3.0 | 9.5 ± 4.3 | 1 |
| Sex (male) | 18 (42%) | 9 (45%) | 3 (25%) | 6 (55%) | 0.26 |
| Underlying disease | 10 (23%) | 4 (20%) | 5 (54%) | 1 (9%) | 0.19 |
| Contact history | 15 | 9 | 2 | 2 | 0.24 |
| Use of antibiotics prior to hospitalization | 20 | 7 | 4 | 9 | 0.42 |
| Hospitalization (d) | 5.7 ± 4.1 | 5.1 ± 2.8 | 7.7 ± 6.2 | 4.7 ± 2.8 | 0.3 |
| ICU admission | 5 (12%) | 1 (5%) | 3 (25%) | 1 (9%) | 0.14 |
| Duration of fever (d) | | | | | |
| Prior to admission | 5.7 ± 3.5 | 6.7 ± 3.2 | 4.7 ± 4.8 | 5.3 ± 1.5 | 0.04 |
| After admission | 2.5 ± 3.0 | 2.4 ± 2.9 | 3.0 ± 4.3 | 2.3 ± 1.3 | 0.25 |
| Total duration | 8.3 ± 4.2 | 9.1 ± 4.3 | 7.7 ± 5.4 | 7.6 ± 1.9 | 0.17 |
| Cases ≥7 d | 27 (63%) | 14 (70%) | 5 (42%) | 8 (73%) | 0.24 |
| Fever duration after first dose of azithromycin | 1.9 ± 2.8 | 1.9 ± 2.7 | 3.9 ± 5.3 | 1.5 ± 1.5 | 0.1 |
| Lab findings (median, range) | | | | | |
| At initial admission | | | | | |
| WBC (/μL) | 7020 (2040—27,260) | 8425 (4810—12,260) | 10,165 (2040—27,260) | 6190 (4270—8790) | 0.37 |
| Platelets (×103/μL) | 248 (128—821) | 266 (178—531) | 279 (162—821) | 192 (128—316) | 0.74 |
| Hemoglobin (g/dL) | 12.7 (8.2—15.3) | 12.7 (8.2—14.5) | 12.6 (8.5—13.6) | 12.6 (11.3—15.3) | 0.37 |
| CRP (mg/dL) | 4.1 (0—24.4) | 4.9 (0—17.2) | 2.7 (0—24.4) | 4.1 (1.5—12.1) | 0.2 |
| During admission | | | | | |
| WBC (/μL) | 11,160 (4930—27,070) | 8805 (5030—27,070) | 13,820 (11, 750—14,860) | 4930 | 0.29 |
| CRP (mg/dL) | 2.7 (1.2—7.4) | 4.6 (1.4—7.4) | 8.0 (2.0—13.0) | 1.8 | 0.29 |
| *M. pneumoniae* IgM titer (BU/mL) | 41.8 (20.3—121.2) | 44.7 (25.5—121.1) | 30.5 (20.2—112.8) | NA | 0.33 |

[a] Cases with coinfections were excluded.
[b] Comparisons were made between PCR(+)/IgM(+) and PCR(−)/IgM(+) groups. Chi-square test or Fisher's exact test was performed for categorical variables and the Mann—Whitney *U* test for continuous variables.
Data are presented as *n* (%) or mean ± SD.
CRP = C-reactive protein; ICU = intensive care unit; IgM = immunoglobulin M; NA = not applicable; PCR = polymerase chain reaction; SD = standard deviation; WBC = white blood cell.

**Table 4** Comparison of clinical parameters between patients with lobar pneumonia and those with bronchopneumonia in 43 patients with positive *Mycoplasma pneumoniae* RT-PCR or IgM

| | Lobar | Bronchopneumonia | *p* |
|---|---|---|---|
| | *n* = 27 | *n* = 16 | |
| Duration of fever (d) | 8.2 ± 3.8 | 8.5 ± 4.8 | 0.88 |
| Hospitalization (d) | 5.3 ± 2.7 | 6.4 ± 5.8 | 0.75 |
| ICU | (3) | (2) | 0.34 |
| Lab findings (median, min–max) | | | |
| WBC (/μL) | 6790 (4270–15,520) | 8975 (2040–27,260) | 0.13 |
| CRP (mg/dL) | 3.8 (0.4–17.2) | 4.7 (0–24.4) | 0.8 |
| *M. pneumoniae* PCR load (copies/mL) | $4.2 \times 10^5$ | $2.9 \times 10^4$ | 0.14 |
| IgM value (BU/mL) | 40.8 (24.7–114.4) | 42.8 (20.3–121.1) | 0.99 |
| Treatment requirement | | | |
| $O_2$ | 2 | 0 | 0.52 |
| Thoracocentesis | 2 | 0 | 0.52 |
| Mechanical ventilation | 1 | 0 | 1 |
| Outcome | | | |
| Complication | 0 | 0 | NA |
| Sequela | 0 | 0 | NA |

Data are presented as *n* (%) or mean ± standard deviation. Patients with detectable coinfections were excluded. CRP = C-reactive protein; ICU = intensive care unit; IgM = immunoglobulin M; NA = not applicable; RT-PCR = real-time polymerase chain reaction; WBC = white blood cell.

or the detection of *M. pneumoniae* DNA by RT-PCR. Overdiagnosis of mycoplasma pneumonia was common. The majority of the patients were treated with azithromycin. Although a proportion of these *Mycoplasma*-negative patients might have been infected by azithromycin-sensitive pathogens such as *Chlamydia* and could still be benefited from macrolide usage, overuse of macrolides is obviously an immediate concern. Reliable, rapid, and easy-to-perform tests for the diagnosis of *M. pneumoniae* infections are helpful in guiding azithromycin usage and preventing further selection of macrolide-resistant *M. pneumoniae*.

A great discrepancy was noted between *M. pneumoniae* IgM and RT-PCR results. Only 12.6% (23/182) of patients who underwent both tests showed positive results in both at the same time. RT-PCR failed to detect *M. pneumoniae* DNA in 47.7% of patients who were positive for *M. pneumoniae* IgM. When RT-PCR was used as a diagnostic standard, a positive *M. pneumoniae* IgM indicated true acute *M. pneumoniae* infection in about half of cases. This might be explained by the fact that *Mycoplasma* IgM can remain detectable for several months after infections. Therefore, detection of *Mycoplasma* IgM does not necessarily confirm acute infection. When patients are tested negative for *M. pneumoniae* IgM, in about 86% of cases they are not truly infected by *M. pneumoniae*. The specificity of *M. pneumoniae* IgM can be elevated further from 85.5% to 90.3% by increasing the cutoff value from 20 BU/mL to 25 BU/mL, but it does not change the sensitivity (62.2%).

Many studies have also compared PCR and serological tests for the diagnosis of *M. pneumoniae*.[5,9,10] Beersma et al[9] evaluated 12 commercial EIA assays by paired sera and used PCR as a gold standard. The sensitivity ranged widely from 35% to 77% and specificity from 49% to 100%. Our results were in line with these earlier reports.

It is well known that a positive conversion or a significant increase in IgG antibody titer in paired sera taken at least 2 weeks apart indicates an acute infection. However, taking two consecutive blood samples 2 weeks apart is too late for guiding treatment decisions. Sometimes, repeated blood sampling is simply impossible in children. Our current study showed that two consecutive blood samples were collected from no more than 10% of pediatric patients with clinical diagnosis of mycoplasma pneumonia in our routine clinical practice.

In addition, our study also confirmed earlier reports that *M. pneumoniae* IgM assay performed in the first week after disease onset frequently yielded negative results.[6,11] Obviously, *M. pneumoniae* IgM titer might have not increased to a detectable level in the first week after fever onset. This phenomenon also hindered early detection, which is highly desired in the clinical setting for guiding treatment. By contrast, the sensitivity of RT-PCR decreases as the duration between symptom onset and sample collection increases.[12]

PCR-based tests have become increasingly important for the early diagnosis of *M. pneumoniae* infections. However, RT-PCR for *M. pneumoniae* infection is not perfect either. Colonization or prolonged shedding from previous infection episodes may account for the *M. pneumoniae* identified by RT-PCR. An earlier study showed that persistent carriage of *M. pneumoniae* DNA in the throat is common following an acute infection.[7] In patients with *M. pneumoniae* infection, *M. pneumoniae* PCR load decreased over time, with a median period of 45–52 days.[7] A threshold of $10^4$ genomic DNA copies per milliliter was proposed to distinguish clinical infection from carriage.[13] However, the cutoff value is not generally accepted. The true prevalence of asymptomatic carriage of *M. pneumoniae* in healthy children is unknown. Studies have detected low *M. pneumoniae* carrier rates of 0.1–13.5% in healthy individuals.[14,15] We cannot exclude

the possibility that some of the *M. pneumoniae* detected by RT-PCR in our study were actually innocent bystanders, and not real pathogens. Therefore, no single test that can be reliable for the diagnosis of *M. pneumoniae* infection is available. A combination of various tests is probably the most reliable approach.[12]

It was reported that the incidence of *M. pneumoniae* infection does not vary greatly with seasons, but the proportion of patients with *M. pneumoniae* pneumonia is greatest during the summer and early fall in regions with temperate climate.[16,17] Our study showed results in line with this finding. The highest numbers of PCR-confirmed cases were observed in July and October in our study.

Pneumonia caused by *M. pneumoniae* is described as walking pneumonia because the patients normally appear relatively well without respiratory distress. Chest radiographic findings were frequently discordant with clinical presentations and lab findings.[18,19] Again, our study results were compatible with previous reports; two-thirds (66%) of our patients showed lung consolidation, but only very few of them had mild tachypnea and leucocytosis. The majority showed no obvious respiratory distress and recovered without any complication. Prolonged fever was not uncommon in our cases. The total fever duration of PCR- or IgM-positive, hospitalized cases in this study was as long as $8.3 \pm 4.2$ days. An earlier report showed that the total fever duration of mycoplasma pneumonia was about 5.5—6.5 days, and older patients tended to have a longer fever duration.[20] Another report showed that children with *M. pneumoniae* infection frequently have a fever duration of 6 days or more prior to admission.[21] In patients with *M. pneumoniae* infection, response to macrolide treatment is usually good. In the current study, fever subsided $1.9 \pm 2.8$ days after the first dose of azithromycin. The reason why fever duration seemed longer in the current study is unclear. In the past few years, the prevalence of macrolide-resistant *M. pneumoniae* has been increasing in Taiwan and elsewhere. This might partly underlie the long fever duration observed in the current study. However, macrolide resistance is beyond the scope of this study.

Interestingly, evidence of lobar pneumonia on chest radiograph and higher bacterial loads detected by RT-PCR were not associated with severity among RT-PCR-positive patients in our study. An earlier report showed that the mean higher *M. pneumoniae* loads were associated with more advanced respiratory disease.[22] In their study, *M. pneumoniae* loads were significantly higher in hospitalized patients than in outpatients. Our analysis focused on hospitalized patients alone and, hence, may have inadequate power to show correlations between bacterial loads and disease severity.

When comparisons were made between patients with positive and negative *M. pneumoniae* PCR among IgM-positive patients, the duration of fever prior to hospitalization was longer and that after hospitalization was shorter in the PCR-positive group, suggesting that hospitalization was delayed in that group. Possible explanations for this are that some of the PCR-positive, IgM-negative patients were actually not infected by *M. pneumoniae*, and also that symptoms of *Mycoplasma* infections are usually mild despite fever. They were seldom hospitalized unless fever persisted for a long time.

Limitations do exist in our current study. First, in a retrospective study like this, timing of blood and throat swab sampling varied greatly from case to case. This might have affected the values of IgM and bacterial loads significantly, making interpretation of these data difficult. Second, having no gold standard for *M. pneumoniae* infection, such as four-fold rise of IgG antibodies in paired serum, is another major limitation of the current study. Using throat swab RT-PCR assay as a gold standard for estimating sensitivity of *M. pneumoniae* IgM is not irrefutable. PCR assays may have false-negative results due to inhibitors in samples, poor technique, or timing of sample collection. Contamination can happen, leading to false-positive results.[4,12,23,24] PCR alone is frequently insufficient for the diagnosis of *M. pneumoniae* infection. Clinically, interpretation of *Mycoplasma* IgM or PCR results should be considered with caution. A combination of PCR plus serology assays as an early diagnostic testing for patients with compatible clinical manifestations may yield the most reliable results.

## Conflicts of interest

All authors declare that they have no conflicts of interest related to the material discussed in this article.

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