Letter to the Editor

Establishment of a Predictive Diagnostic Model for Acute Mycoplasma Pneumoniae Infection in Elderly Patients with Community-Acquired Pneumonia

XIAO Hong Li¹, XIN De Li², WANG Yan¹, CUI Li Jian³, LIU Xiao Ya⁴, LIU Song⁵, SONG Li Hong⁶, LIU Chun Ling⁷, and YIN Cheng Hong⁴,*

We established a diagnostic model to predict acute Mycoplasma pneumoniae (M. pneumoniae) infection in elderly CAP patients. We divided 456 patients into acute and non-acute M. pneumoniae infection groups. Binary logistic regression and receiver operating characteristic (ROC) curves were used to establish a predictive model. The following independent factors were identified: age ≥ 70 years; serum cTNT level ≥ 0.05 ng/mL; lobar consolidation; mediastinal lymphadenopathy; and antibody titer in the acute phase ≥ 1:40. The area under the ROC curve of the model was 0.923 and a score of ≥ 7 score predicted acute M. pneumoniae infection in elderly patients with CAP. The predictive model developed in this study has high diagnostic accuracy for the identification of elderly acute M. pneumoniae infection.

Key words: Elderly; Community-acquired pneumonia; Mycoplasma pneumoniae

Community-acquired pneumonia (CAP) is an important cause of morbidity and mortality, especially in patients admitted to the hospital[1]. Previous epidemiological surveys of adult CAP patients in China and Asia have suggested that Mycoplasma pneumoniae (M. pneumonia) was the main causative agent of CAP[2]. M. pneumoniae infection is commonly encountered in young adults. However, there has been a gradual increase in the number of elderly patients with M. pneumoniae infection[3]. Very few studies have explored the clinical characteristics of elderly patients with CAP with acute M. pneumoniae infection. Therefore, in the present study, we analyzed the prevalence and the clinical and radiological characteristics of acute M. pneumoniae infection in elderly CAP patients hospitalized in China. Furthermore, we established a diagnostic model to detect acute M. pneumoniae infection in elderly patients.

Patients A total of 456 elderly patients with CAP, who were admitted to the Department of Respiration, Emergency Department, and the Department of Infectious Disease of three hospitals in Beijing between August 2011 and December 2015, were recruited to this cross-sectional, observational, multicenter study. These hospitals were the Beijing Friendship Hospital, Air Force General Hospital, PLA, and Beijing Guang Wai Hospital.

The following inclusion criteria were applied. (1) age ≥ 60 years; (2) CAP diagnosis, which was defined as the presence of new infiltrates on chest X-ray and the presence of at least one of the following clinical features: a newly developed or exacerbated cough with or without sputum production, fever (body temperature > 37.8 °C) or hypothermia (body temperature < 35.6 °C), leukocytosis (defined as a leukocyte count > 10 × 10⁹ cells/L), leukopenia (defined as a leukocyte count < 4 × 10⁹ cells/L)[4]; (3) the patients agreed to participate in this study and voluntarily accepted the diagnostic tests. Patients who were pregnant, lactating, diagnosed positive for human immunodeficiency virus infection, had clinical symptoms for more than 1 week, hospital-acquired pneumonia or Mycoplasma pneumoniae infection were excluded.

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pneumonia, tuberculosis, lung tumors, aspiration pneumonia, an organ transplant, and/or were treated with immunosuppressive drugs were excluded from study participation.

The study protocol was approved by the Ethics Committees of all three participating hospitals.

Data collection methods Patients’ clinical data were prospectively collected using a identical case observation form and included demographic factors, symptoms other signs (moist and dry rales), laboratory data, and chest radiographic data.

Specimen collection For specimen collection, 5-10 mL of venous blood samples were collected from patients 1 day after enrolment prior to antibiotic treatment and then at two-week intervals thereafter. Serum samples were prepared by centrifugation at 3000 x g for 10 min. One day after enrolment, sterile throat swabs (167KSO1, Guangzhou, China) were collected from each patient, immediately soaked in 2 mL physiological saline, and all collected samples were stored at -80 °C within 24 h, until required.

Serological detection Serum specimens obtained from the acute and convalescence phases were selected for testing of M. pneumoniae infection using a passive agglutination test (Serodia-Myco II, Fujirebrio Inc., Japan). This detects the mixed antibody titers of M. pneumoniae IgG, IgA, and IgM.

Detection of M. pneumoniae DNA. Nested PCR was used to detect M. pneumoniae DNA in throat swabs in the Clinical Medicine Research Institute of the Beijing Friendship Hospital, Capital Medical University.

DNA extraction Throat swab specimens were collected and centrifuged at 12, 000 x g for 10 min, and the supernatant was removed. Then, 50 µL of 1% Triton X-100 was added to the pellet, re-suspended, and placed in a boiling water bath for 10 min.

PCR amplification and DNA sequencing The 23S rRNA M. pneumoniae primer sequences were retrieved from the National Center for Biotechnology Information (NCBI, USA, www.ncbi.nlm.gov/nucleotide). Primers were designed for amplification of the 23S rRNA gene (Outer primer: P1 5′-GGTC CTAAGGTAGCCGAATT-3′ and P2 5′-CAGTIAACCA AATACAGAG-3′; product length 292 base pair; Inner primer: P3 5′-CCTAGTCGGTGATAATCCT-3′ and P4 5′-CCAAGGGTAGTATTCCACCT-3′, product length 239 base pair) according to our previous research reports.[14] The first PCR cycle was conducted as follows: 94 °C for 2 min; followed by 93 °C for 1 min, 50 °C for 1 min, and 72 °C for 30 cycles; and a final 5-min extension cycle at 72 °C with P1 and P2 as primers. The second amplification was conducted using the products of the first PCR reaction as a template, using primers P3 and P4, and with the same cycle conditions. The resultant products were electrophoresed to detect the target fragment. With regard to the specimens that tested positive by electrophoresis, the second amplified products were purified and subjected to full automated DNA sequencing in an ABI 3730XL sequencer (Shanghai Sangon Biological Technologies & Service Co., Ltd). The resulting sequences were compared with the corresponding sequences of the standard M129 strain registered at NCBI.

Serological and PCR diagnosis Patients were diagnosed as having an initial acute M. pneumoniae infection if: the mixed antibody titer of the convalescence period increased four-fold or more relative to the acute phase; the mixed antibody titer of the acute phase was less than 1:40 and that of the convalescence period was greater than 1:80; and the mixed antibody titer of the acute and convalescence phases exceeded 1:160 and the DNA test by PCR was positive. Patients were diagnosed as having repeat M. pneumoniae infection if the mixed antibody titer of the acute and convalescence phases all exceeded 1:160 but the DNA test was negative. Patients were diagnosed as carriers of M. pneumoniae if the DNA test was positive but the serological results did not meet the requirements of acute infection or previous exposure.[5]

Statistical methods Data were analyzed by SPSS, version 16.0. Patients were divided into two groups based on the type of clinical infection: acute and non-acute M. pneumoniae infection. The latter group included individuals who were negative for M. pneumoniae infection, those with a history of infection, and carriers. First, the demographic factors, symptoms, signs, and laboratory data between the acute and non-acute cases were compared. For measurement data, if the data were normally distributed, the Student’s t test was used and the data were expressed as mean ± standard deviation (SD), if the data were not normally distributed, the non-parametric Mann-Whitney U test was used and the data were expressed as Median (QuartileLlow, QuartileU). For count data, the χ2 test was used. Second, the independent risk factors were identified by binary logistic regression and estimated by the odds ratio (OR), then weighted according to the regression coefficient and formulated into a predictive diagnostic model. Finally, the diagnostic
accuracy was calculated using the receiver operating characteristic (ROC) curves. A probability of \( P < 0.05 \) was considered statistically significant.

**Prevalence of M. pneumoniae infection in elderly patients with CAP** A total of 456 hospitalized elderly patients with CAP were recruited to this study. The median age of the patients was 76.5 ± 8.6 (range, 60-97 years), and 53.1% were male. Of these, 284 were identified as being positive for *M. pneumoniae* by PCR, and the percentage positive was 62.3% (Figure 1A). The major therapeutic drugs for *M. pneumoniae* infection are macrolide antibiotics. Recent research has shown that the macrolide resistance rates in *M. pneumoniae* in Beijing were as high as 98.4%, 95.4%, and 97.0% in 2010, 2011, and 2012, respectively[^6]. Such resistance has been confirmed to have occurred as a result of a point mutation in the 23S rRNA gene, especially in loci 2063 and 2064[^6]. In our study, of the 284 positive cases, 146 (51.4%) were infected with mutant strains of *M. pneumoniae* with the following mutations: A2063G (92 cases), presence of both A2063G and A2064G (44 cases), and A2064G (10 cases; Figure 1B-D). The results indicated that at least half of the cases of CAP due to *M. pneumoniae* would be resistant to erythromycin, and the common mutation sites were A2063G and A2064G, which corroborate previous reports.

Diagnosis of *M. pneumoniae* depends on serological tests or molecular detection and/or microbial culture. However, these methods have some limitations. Culture methods are time-consuming, the sensitivity of serological tests are uncertain, and PCR tests can yield both false negative and false positive results. In our study, we combined serological and PCR methods, which are good screening tests, to accurately and reliably diagnose *M. pneumoniae* infection[^5]. Among the positive cases, 48 (10.5%) had acute *M. pneumoniae* infection, which is similar to rates of 13.3–13.5% previously reported by Liu et al[^7]. A multicent epidemiological study of CAP by *M. pneumoniae* in adults in Asia between 2001 to 2002 showed that the prior *M. pneumoniae* infection and carrier rate was 5.7%^[^5]. Different from that, in the present study, 240 patients (52.6%) were carriers of *M. pneumoniae*, among which 120 were carriers of mutant strains (mutation rate, 50.0%). There were 4 patients (0.9%) with previous infection and 164 CAP cases (36.0%) that were considered negative for *M. pneumoniae* infection. This indicates that the number of carriers has increased dramatically in recent years, especially in elderly patients.

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**Figure 1.** (A) Electrophoretogram of the partial PCR products of domain V of 23S rRNA of the strain. FH, International standard strain of *M. pneumoniae*; N, Negative control; 127-132, Sample number. (B) FH (no site mutation). The red underlined color indicates the bases in the target sites 2063 and 2064 of the 23S rRNA of the standard strain targeted by erythromycin. All targeted bases were A. (C) Clinically isolated MP strains (A2063G site mutation). The red underline represents the base mutation (A→G) at site 2063. (D) Clinically isolated MP strains (A2064G site mutation). The red underline represents the base mutation (A→G) at site 2064.
**Patient demographic and clinical features** The patients were divided into two groups based on the type of infection: 48 had acute *M. pneumoniae* infection and 408 had non-acute *M. pneumoniae* infection. Patients with CAP due to acute *M. pneumoniae* infection were significantly older than those with non-acute *M. pneumonia* infection (79 years vs. 76 years, *P* < 0.05). Autoimmune reactions are known to be responsible for many of the extrapulmonary complications of *M. pneumoniae* infection[8]. In line with this results, we found that acute *M. pneumoniae* infection was associated with autoimmune disease (*P* < 0.05, Supplemental Table 1, available in www.besjournal.com). The clinical symptoms and signs did not significantly differ between the two groups. In addition, previous studies have reported that the prevalence of cardiac complications ranged from 1 to 8.5%, but is more common in adults than in children[8]. In our study, the rate of increased serum cTNT level in the acutely infected group was higher than that in the non-acutely infected group [41.7% (20/48) vs. 23.5% (96/408); *P* = 0.006], indicating that myocardial injury was more common in elderly patients with CAP due to *M. pneumoniae*. The antibody titer in the acute phase exceeded 1:40 in significantly more patients with acute infection than with non-acute infection (*P* < 0.05; Supplemental Table 2, available in www.besjournal.com).

**Radiographic features of patients with acute infection** In adults, computed tomography (CT) findings associated with *M. pneumoniae* infection show areas of diffuse and/or multifocal lesions, ground-glass opacities, lobular consolidation, centrilobular nodules, and hilar lymphadenopathy[8]. In this study, diffuse lesions in the bilateral lungs (45.8% vs. 66.7%, *P* < 0.05), lobular consolidation (27.5% vs. 50.0%, *P* < 0.05), photic zone (15.2% vs. 29.2%, *P* < 0.05), hilar lymphadenopathy (16.7% vs. 45.8%, *P* < 0.05), and mediastinal lymphadenopathy (29.4% vs. 70.8%, *P* < 0.05) were more common in the acutely infected group than in the non-acutely infected group. Furthermore, lesions in the lower left lung lobe were less common (37.3% vs. 20.8%, *P* < 0.05) in the acutely infected group than in the non-acutely infected group (Supplemental Table 3, available in www.besjournal.com).

**Predictive diagnostic model of acute *M. pneumoniae* infection.** We identified the following independent factors associated with acute *M. pneumoniae* infection: age ≥ 70 years (*OR* 12.03), serum cTNT level ≥ 0.05 ng/mL (*OR* 5.77), lobular consolidation (*OR* 2.54), mediastinal lymphadenopathy (*OR* 7.47), and antibody titer in the acute phase ≥ 1:40 (*OR* 36.83; Table 1). Based on these factors, we developed a predictive diagnostic model of acute *M. pneumoniae* infection in the elderly (Table 2). The area under the ROC curve (AUC) of the model was 0.923, and the standard error was 0.019 with a 95% CI of 0.885-0.961. A score of 7 alerted doctors to elderly CAP patients with acute *M. pneumoniae* infection (85.3% specificity and 83.3% sensitivity); consequently, 83.3% of patients with acute *M. pneumoniae* infection would have a score of ≥ 7, while 16.7% of patients with acute *M. pneumoniae* infection would have a lower score.

### Table 1. Independent factors for acute *M. pneumoniae* infection

<table>
<thead>
<tr>
<th>Factors</th>
<th>B¹</th>
<th>sx²</th>
<th>P</th>
<th>B'³</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>2.487</td>
<td>0.817</td>
<td>0.002</td>
<td>23.92</td>
<td>4</td>
</tr>
<tr>
<td>cTNT</td>
<td>1.753</td>
<td>0.448</td>
<td>0.000</td>
<td>9.24</td>
<td>2</td>
</tr>
<tr>
<td>Lobar consolidation</td>
<td>0.933</td>
<td>0.446</td>
<td>0.036</td>
<td>4.90</td>
<td>1</td>
</tr>
<tr>
<td>Mediastinal lymphadenopathy</td>
<td>2.011</td>
<td>0.461</td>
<td>0.000</td>
<td>10.91</td>
<td>2</td>
</tr>
<tr>
<td>Antibody titer in acute phase</td>
<td>3.606</td>
<td>0.483</td>
<td>0.000</td>
<td>20.50</td>
<td>4</td>
</tr>
</tbody>
</table>

**Note.** ¹Partial regression coefficient; ²Standard error; ³Standard regression coefficient.

### Table 2. Predictive diagnostic model of acute *M. pneumoniae* infection in elderly patients with CAP

<table>
<thead>
<tr>
<th>Items</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60-70</td>
<td>71-80</td>
<td>&gt; 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cTNT</td>
<td>&lt; 0.05</td>
<td>≥0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobar consolidation</td>
<td>No</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinal lymphadenopat-hy</td>
<td>No</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody titer in acute phase</td>
<td>&lt; 1:40</td>
<td>1:40-1:79</td>
<td>1:80-1:159</td>
<td>≥ 1:160</td>
<td></td>
</tr>
</tbody>
</table>
Only 14.7% of patients with non-acute infection scored 7 or higher. Previous studies have developed a diagnostic model to differentiate between pneumonia caused by *M. pneumoniae* from that by other etiological agents, such as the Japanese Respiratory Society CAP guidelines\(^9\). However, this system was unable to identify many cases of acute *M. pneumoniae* infection in elderly patients with several underlying diseases\(^9\). Liu et al.\(^10\) developed a CAP diagnostic model in adult outpatients with *M. pneumoniae* infection in Beijing with an AUC of only 0.61. In this study, we established a diagnostic model of acute *M. pneumoniae* infection, the parameters of which could be attained during the early stage of the disease. The model developed in the present study has a high accuracy.

This study had several limitations. The study has a relatively small sample size. Furthermore, data from elderly outpatients were not collected. Finally, we did not validate this scoring system using a larger number of elderly patients with CAP. Further studies with a larger sample size conducted over a longer term are warranted.

In summary, the predictive diagnostic model developed in the current study can differentiate acute *M. pneumoniae* infection from negative and previous infection and pathogen carriers, with high diagnostic accuracy among elderly individuals in the early stages of CAP.

**ACKNOWLEDGEMENTS**

We are grateful to Tianhao Su for radiographic analysis and Yuanyuan Kong for analysis of the data.

**AUTHOR CONTRIBUTIONS**

CHY and HLX designed this study. YW, LJG, XYL, LHS and CLL recruited participants and SL screened participants for study entry; DLX conducted the serological and PCR tests. CHY and HLX analyzed data and wrote the manuscript and subsequently all authors provided advice and approved the final manuscript.

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**REFERENCES**

### Supplemental Table 1. Demographic factors of patients with acute *M. pneumoniae* infection

<table>
<thead>
<tr>
<th>Factors</th>
<th>Acute <em>M. pneumoniae</em> infection (n = 48)</th>
<th>Non-acute <em>M. pneumoniae</em> infection (n = 408)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) ≥ 70 (%)</td>
<td>46 (95.8)</td>
<td>310 (76)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Chronic renal insufficiency (%)</td>
<td>2 (4.2)</td>
<td>60 (14.7)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Autoimmune disease (%)</td>
<td>4 (8.3)</td>
<td>8 (2.0)</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

### Supplemental Table 2. Laboratory test results of patients with acute *M. pneumoniae* infection

<table>
<thead>
<tr>
<th>Items</th>
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<th>Non-acute <em>M. pneumoniae</em> infection (n = 408)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac troponin T [ng/mL, M(Q_L, Q_U)]</td>
<td>0.00 (0.00, 0.05)</td>
<td>0.00 (0.00, 0.00)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Antibody titer of acute phase ≥ 1:40</td>
<td>28 (58.3)</td>
<td>34 (8.3)</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

**Note.**  M (Q_L, Q_U), Median (Quartile_Low, Quartile_Up); CK-MB, Creatine kinase MB isoenzyme; PT, prothrombin time; APPT, activated partial thromboplastin time.

### Supplemental Table 3. Radiographic characteristics of patients with acute *M. pneumoniae* infection

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Acute <em>M. pneumoniae</em> infection (n = 48)</th>
<th>Non-acute <em>M. pneumoniae</em> infection (n = 408)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower lobe of the left lung (%)</td>
<td>10 (20.8)</td>
<td>152 (37.3)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Diffuse lesions of bilateral lungs (%)</td>
<td>32 (66.7)</td>
<td>186 (45.6)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Lobar consolidation (%)</td>
<td>24 (50.0)</td>
<td>112 (27.5)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Photic zone (%)</td>
<td>14 (29.2)</td>
<td>62 (15.2)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Hilar lymphadenopathy (%)</td>
<td>22 (45.8)</td>
<td>68 (16.7)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Mediastinal lymphadenopathy (%)</td>
<td>34 (70.8)</td>
<td>120 (29.4)</td>
<td>&lt; 0.05</td>
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</tbody>
</table>