Letter to the Editor

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

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We established a diagnostic model to predict acute *Mycoplasma pneumoniae (M. pneumonia)* infection in elderly Community-acquired pneumonia (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.

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 aggravated 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 > 10×10^9 cells/L), leukopenia (defined as a leukocyte count < 4×10^9 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

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symptoms for more than 1 week, hospital-acquired 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 3,000 ×*g* for 10 min. One day after enrolment, sterile throat swabs (167KS01, 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 ×*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 CTAAGGTAGCGAAATT-3' and P2 5'-CAGTIACCAA TTAGAACAGC-3', product length 292 base pair; Inner primer: P3 5'-CCTAGTCGGGTAAATTCCGT-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 skewed distributed, the non-parametric Mann-Whitney *U* test was used and the data were expressed as Median ($Quartile_{Low}$, $Quartile_{Up}$). For count data, the χ^2 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