The Brazilian Journal of
INFECTIOUS DISEASES
www.elsevier.com/locate/bjid

Original article

Clinical significance of different bacterial load of *Mycoplasma pneumoniae* in patients with *Mycoplasma pneumoniae* pneumonia

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ARTICLE INFO

Article history:
Received 3 April 2013
Accepted 12 June 2013
Available online 26 October 2013

Keywords:
Mycoplasma pneumoniae
Pneumonia
Polymerase chain reaction
Serology

ABSTRACT

Objective: This retrospective study was conducted to investigate the clinical significance of different *Mycoplasma pneumoniae* bacterial load in patients with *M. pneumoniae* pneumonia (MP) in children.

Methods: Patients with MP ($n = 511$) were identified at the Children's Hospital Affiliated to Soochow University database during an outbreak of MP between January 2012 and February 2013.

Results: Comparing patients with high and low bacterial load those with higher loads were significantly older ($p < 0.01$) and had fever significantly more frequently ($p = 0.01$). Presence of wheezing at presentation was associated with low bacterial load ($p = 0.03$). Baseline positive IgM was present in 93 (56.4%) patients with high bacterial load compared to 46 (27.8%) patients with low bacterial load ($p < 0.001$). Co-infection with viruses was found significantly more frequent among patients with low bacterial load (24.2%) than those with high bacterial load (8.5%) [$p < 0.001$]. Bacterial co-infection was also more frequently detected among patients with low bacterial load (22.4%) than in those with high bacterial load (12.1%) [$p = 0.01$].

Conclusion: *M. pneumoniae* at a high bacterial load could be an etiologic agent of respiratory tract disease, whereas the etiologic role of MP at a low bacterial load remains to be determined.

Introduction

Mycoplasma pneumoniae is an important pathogen responsible for community-acquired pneumonia in children. *M. pneumoniae* pneumonia (MP) has been reported in 10–40% of community-acquired pneumonia cases.[1–4] The mainstay for diagnosing of *M. pneumoniae* infection presently relies on a rise in paired titer by serological methods.[5] Polymerase chain reaction (PCR) has been applied for the early detection

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1413-8670
http://dx.doi.org/10.1016/j.bjid.2013.06.004

of *M. pneumoniae* infection.[6,7] The role of PCR assays performed on upper respiratory tract samples for the diagnosis of *M. pneumoniae* infection is controversial.[5,8] Previous study demonstrated that PCR can detect persistent *M. pneumoniae* infection up to 7 months after disease onset, the bacterial load in consecutive samples gradually declined in relation to the time interval from onset of illness to sampling.[9] Thus, the clinical significance of different *M. pneumoniae* bacterial load is of much interest.

This study was conducted to investigate the clinical significance of different *M. pneumoniae* bacterial load using nasopharyngeal aspirates (NPAs) during a recent outbreak of MP in children.

Materials and methods

Patients and specimens

This study was a retrospective analysis of *real-time* PCR for diagnostic testing of *M. pneumoniae* infection by antigen detection. Patients hospitalized with clinically and radiologically confirmed lower respiratory tract infections ($n = 1429$) were identified at the Children's Hospital Affiliated to Soochow University database during an outbreak of MP between January 2012 and February 2013. Both serum and NPAs were obtained from these patients. Therefore, patients without stored serum samples or results of NPAs were excluded from our study. Paired serum samples taken on admission and at least seven days after the first collection were available for 733 patients (51.3%).

Real-time PCR

Nasopharyngeal swabs were obtained within 24 h of admission. The specimens were centrifuged and were stored at −80 °C until tested. A quantitative diagnostic kit (DaAn Gene Co., Ltd., Guangzhou, China) for *M. pneumoniae* DNA was used to determine the load of MP, as previously reported.[10] The method is based on the TaqMan PCR technology, and the target is 16S rRNA gene specific for MP genome. Briefly, 1 mL of nasopharyngeal aspirates diluted with 4% NaOH was centrifuged at 12,000 rpm for five min. The sediment was collected, washed twice with 0.9% NaCl, blended with 50 μL of DNA extraction solution, incubated at 100 °C for 10 min, and centrifuged at 12,000 rpm for five min. Real-time PCR was performed on the resulting supernatant with 2 μL, and PCR mix with 43 μL (supplied with the kits) using the DA 7600 real-time PCR system (Applied Biosystems, California, USA) as follows: 93 °C for 2 min, 10 cycles of 93 °C for 45 s and 55 °C for 60 s, followed by 30 cycles of 93 °C for 30 s and 55 °C for 45 s. All nasopharyngeal swabs were tested for antigen detection by immunofluorescence for seven common viruses (RSV, adenovirus, influenza viruses A and B, and parainfluenza viruses 1, 2 and 3).

Serology

Detection of serum *M. pneumoniae*-specific antibody was performed using enzyme-linked immunosorbent assay (Virion-Serion, Germany). The assay was considered positive if IgM ≥ 1.1 or if there was ≥4-fold rise in IgG titer.[8]

Diagnosis of MP

Diagnosis of MP was based on serology or PCR findings. A significant rise in *M. pneumoniae* IgG titer or the presence of IgM antibodies was used as criteria for current *M. pneumoniae* infection. Likewise, DNA detection by real-time PCR was also considered *M. pneumoniae* infection.

Review of medical records

Data compiled from a retrospective chart review of all study patients were extracted through the use of specially prepared data forms without knowledge of serologic status or PCR results. The information extracted included demographics, clinical features and laboratory data.

Statistical analysis

We used *n* (%) for categorical variables and median (IQR) for continuous variables with non-normal distribution or mean (SD) for those with normal distribution. We assessed differences in categorical variables with the χ^2 test. We calculated 95% CI for differences in medians with an exact test. Logistic regression analysis was performed to identify clinical characteristics associated with different bacterial loads. SPSS (version 17.0) software was used for all statistical analysis.

Results

Demographic findings

Among 1429 patients with lower respiratory tract infections, 511 (35.8%) were diagnosed with MP based on serology or PCR findings. Of the 511 patients with MP, 304 (59.5%), the mean age was 35 months (range, one month to 156 months). The age distribution of the patients is shown in Fig. 1. The remaining 918 patients who were not diagnosed with MP did not differ significantly from patients with MP in terms of sex and age at disease onset.

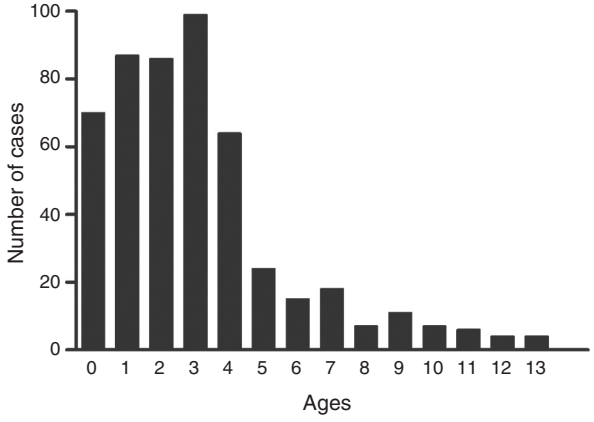

Fig. 1 – Age distribution of our patients.