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# Clinical and laboratory profiles of refractory *Mycoplasma pneumoniae* pneumonia in children

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SUMMARY

*Objectives:* The purpose of this study was to explore the clinical and laboratory characteristics of children with refractory *Mycoplasma pneumoniae* pneumonia (RMPP).
*Methods:* Seventy-six children with RMPP and 26 children with non-refractory *M. pneumoniae* pneumonia (NRMPP), confirmed by both serology and fluorescent quantitation PCR in bronchoalveolar lavage fluid (BALF), were evaluated retrospectively.
*Results:* Compared to those with NRMPP, children with RMPP were older ($66.6 \pm 39.0$ vs. $48.4 \pm 35.4$ months, $p = 0.038$) and had a longer duration of fever ($12.7 \pm 2.6$ vs. $7.5 \pm 1.8$ days) and hospital stay ($12.1 \pm 3.2$ vs. $7.4 \pm 2.9$ days). Children with RMPP presented neutrophil infiltration both in serum and BALF, as well as severe pulmonary lesions with pleural effusion. Children with RMPP had a significantly higher *M. pneumoniae* DNA load in BALF compared to NRMPP patients, and the *M. pneumoniae* load in BALF was significantly correlated with neutrophils and inversely correlated with macrophages for both the NRMPP and RMPP groups. The serum concentrations of tumor necrosis factor alpha (median 114.5 pg/ml, range 49.1–897.9 pg/ml) and interferon gamma (median 376.9 pg/ml, range 221.4–1997.6 pg/ml) were significantly higher in children with RMPP compared to children with NRMPP.
*Conclusions:* This study indicates that a direct microbe effect and the subsequent induced excessive host immune response contribute in part to the progression of RMPP.


## 1. Introduction

*Mycoplasma pneumoniae* is one of the most important pathogens causing community-acquired pneumonia in adults and children.[1,2] Cyclical outbreaks of *M. pneumoniae* infection can be expected on average every 3–7 years, but at any given time *M. pneumoniae* may account for as many as 40% of community-acquired pneumonia cases and 18% of these patients require hospitalization.[3]

*M. pneumoniae* infection is usually a self-limited disease. However, previous studies have shown that *M. pneumoniae* infection can develop into a severe life-threatening disease in rare cases, such as refractory *M. pneumoniae* pneumonia (RMPP), acute respiratory distress syndrome, necrotizing pneumonitis, and fulminant pneumonia.[4–7] Severe *M. pneumoniae* pneumonia appears to occur in defined age groups, whereas older children aged 5–15 years are more likely to develop bronchopneumonia involving one or more lobes.[3]

Generally speaking, drug resistance in *M. pneumoniae* can affect the length and severity of illness in patients with pneumonia. In recent years, the emergence of macrolide-resistant *M. pneumoniae* in epidemic, country-wide, and large community settings has been reported worldwide.[1,8–10] In China, macrolide-resistant *M. pneumoniae* is very common and the prevalence ranges from 83% to 95%.[11,12] However, the clinical relevance of resistant strains in RMPP is still the subject of debate worldwide because of a lack of conclusive evidence.

Besides macrolide resistance, excessive inflammation induced by the host's cell-mediated immunity also contributes to the

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development of the pulmonary lesions caused by *M. pneumoniae*. This may be associated with the progression of *M. pneumoniae* pneumonia, with infiltration of neutrophils and lymphocytes and excessive cytokines or chemokines.[13] Excessive inflammation may play a more important role in RMPP than macrolide resistance in China because of the high prevalence of macrolide-resistant *M. pneumoniae*. Several recent studies have focused on the effectiveness of treatment with corticosteroids in children with RMPP; it was found that a macrolide combined with corticosteroids constituted a better treatment option for children with RMPP than the use of a macrolide alone.[7,14,15] However, further studies are needed to elucidate the associations between host cell-mediated immunity and the progression of RMPP.

In this study, the clinical and laboratory characteristics of children with RMPP were investigated, including the relationship between concentrations of *M. pneumoniae* DNA and cytology in bronchoalveolar lavage fluid (BALF) and cytokines in serum.

## 2. Materials and methods

### 2.1. Study design

This retrospective, single-center study was conducted in the children's hospital affiliated with Soochow University, China, from October 2012 to September 2013. Children were eligible for enrollment if they were aged 4 weeks to 14 years, had a preceding fever, and had clinical (tachypnea, chest retractions, or abnormal findings on auscultation) and radiological evidence of community-acquired pneumonia.

A total of 102 children hospitalized with a final diagnosis of *M. pneumoniae* pneumonia and with *M. pneumoniae* infection confirmed in BALF and serum samples using PCR and ELISA were enrolled. Among these 102 children, 76 were diagnosed with RMPP and 26 with non-refractory *M. pneumoniae* pneumonia (NRMPP). RMPP was diagnosed in the presence of a prolonged high degree fever (>38.5 °C) and radiological deterioration after therapy with a macrolide combined with oral prednisolone (azithromycin 10 mg/day and prednisolone 1–2 mg/day) for 7 days or more.[15] Other children were defined as having NRMPP. In general, a second X-ray was performed in all children after 5–7 days of treatment to evaluate radiological changes; radiological deterioration was defined as an increased area of infiltration or pleural effusion compared to the first radiological findings. Children were excluded if they had a proven immunodeficiency, immunosuppression, chronic lung disease, or asthma. This study was conducted with the approval of the Institutional Human Ethics Committee of Soochow University.

### 2.2. Patient evaluation

Upon hospital admission and at patient discharge, pediatricians completed a questionnaire to collect information on the age and sex of the patient, the duration of symptoms, the clinical symptoms, the laboratory findings, and the effectiveness of treatment with macrolide (azithromycin 10 mg/kg/day once daily for 5–7 consecutive days). Chest radiography was performed using standard equipment and radiographic techniques, and all children enrolled presented unequivocal focal or segmental consolidation with or without pleural effusion on chest radiography. Peripheral blood samples were obtained on admission for the determination of the complete blood count, C-reactive protein concentrations, platelets, immunoglobulins, subpopulations of T lymphocytes, and specific antibody to *M. pneumoniae*. For 15 children with NRMPP and 18 children with RMPP, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), interleukin (IL)-4, and IL-10 were detected by ELISA, as described previously.[16] All children enrolled were treated with alveolar irrigation and drainage using a fiberoptic bronchoscope.[17]

### 2.3. M. pneumoniae *serology*

The specific IgM and IgG antibodies against *M. pneumoniae* were detected in 2 ml of acute phase (on admission) and convalescent phase (on discharge) patient serum using a commercial ELISA kit (SERION ELISA classic *M. pneumoniae* IgG/IgM; Institute Virion/Serion, Würzburg, Germany), in accordance with the manufacturer's instructions and as described previously.[18]

### 2.4. BALF collection

The procedure for BALF collection was performed as described previously.[17] The first BALF sample was used for the detection of *M. pneumoniae* DNA and nine common viruses. The second and third samples were collected and centrifuged at 200 × *g* for 10 min at 4 °C. Cells were counted after Giemsa and Wright staining.

### 2.5. Detection of nine common viruses

A total 2 ml of the first BALF sample was centrifuged at 500 × *g* for 10 min and resuspended in 2 ml saline. It was then divided equally into two aliquots for pathogen detection by direct immunofluorescence assay and PCR, as described previously.[19] One of the samples was analyzed for seven common viruses including respiratory syncytial virus, influenza virus types A and B, parainfluenza virus types 1–3, and adenovirus by direct immunofluorescence assay. The other sample was analyzed for human metapneumovirus and human bocavirus using reverse transcription PCR and real-time PCR.

### 2.6. Fluorescent quantitation (FQ) PCR for M. pneumoniae gene detection

A 16S rRNA gene PCR procedure was used for the detection of *M. pneumoniae*. In brief, one of the equally divided samples of BALF was shaken for 30 s and centrifuged at 15 000 × *g* for 5 min. The sediment was collected and DNA extracted from a 400-μl sample in accordance with the manufacturer's instructions. The DNA was then amplified using PCR primers and probes. Primers and probes were synthesized using the following sequences: *M. pneumoniae*-F: 5′-GCAAGGGTTCGTTATTTG-3′; *M. pneumoniae*-R: 5′-CGCCTGCGCTTGCTTTAC-3′ (344 bp); *M. pneumoniae*-probe: 5′-AGGTAATGGCTAGAGTTTGACTG-3′ (141 bp). FQ-PCR was performed using a iQ5TM BIO-iCycler (Bio-Rad, California, USA), and the cycling conditions were as follows: 2 min at 37 °C; 10 min at 94 °C, and 40 cycles of 10 s at 94 °C, 30 s at 55 °C, and 40 s at 72 °C. Quantification curves were plotted using several concentrations of standard control samples, which were purchased from Daan Gene Co. Ltd (Guangzhou, China). For each assay, a negative quality control, a critical quality control, a positive quality control, and four positive quantity controls ($10^5$ copies/ml, $10^6$ copies/ml, $10^7$ copies/ml, and $10^8$ copies/ml) were used.

### 2.7. Statistical analyses

Numeration data were analyzed using the Chi-square test or Fisher's exact test and measurement data were analyzed using the Student *t*-test or Mann–Whitney *U*-test if the data distribution was non-normal. The Pearson or Spearman correlation test was used to assess correlations among laboratory parameters. Because of the impact of age on peripheral neutrophils, a general linear model was used for this analysis. A two-sided *p*-value of $<0.05$ was considered