1. Background

*M. pneumoniae* is one of the major pathogens of community-acquired pneumonia in children all over the world (1-3). *M. pneumoniae* easily attaches to ciliated epithelial cells of the respiratory tract and causes damage (4, 5). For diagnosis of *M. pneumoniae*, culture and serological diagnosis are common methods in clinical practice, but insensitivity, time-consuming or cross-reactions limit their application (6-10). Recently, PCR method was used to directly detect *M. pneumoniae* DNA, which has high specificity and sensitivity in clinical detection of *M. pneumoniae* (11, 12). But in clinical practice, co-infections are common in children with pneumonia and direct detection of *M. pneumoniae* is not efficient in distinguishing it from pneumonia (8). Our previous study suggested that Th1/Th2 balance plays a significant part in anti-infectious immunity and Th1/Th2 cytokines are useful biomarkers in diagnosis and treatment of bacterial and viral infection (13-15).

2. Objectives

In this study, we wanted to confirm the value of Th1/Th2 cytokines in diagnosis and treatment of *M. pneumoniae* pneumonia.

3. Patients and Methods

Subjects: From May 2012 to June 2014, a total of 13161 throat swab specimens were collected. The age of the 13161 patients ranged from 3 months to 10 years. All these children had been primarily diagnosed with pneumonia (16) and had received no clinical treatment.

Detection of *M. pneumoniae*: *M. pneumoniae* PCR kit (Daan Gen Co., Ltd. Guangzhou, China) was used in DNA extraction and *M. pneumoniae* DNA detection (4). Amplification and data analysis were carried out with an applied biosystems 7500 real-time PCR system (Applied Biosystems, Inc., CA, USA) under this condition: 93°C for 2 minutes and 40 cycles of 93°C for 45 seconds and 55°C for 60 seconds. Specific tests were used to detect other pathogens, such as immunofluorescence to detect respiratory viruses including adenovirus, human metapneumovirus, respiratory syncytial virus, parainfluenza virus and influenza virus; blood and sputum culture for bacteria; and real-time PCR to detect human cytomegalovirus, Epstein-Barr virus, *Chlamydia pneumoniae*, *Ureaplasma urealyticum* and *Chlamydia trachomatis*. If any of these assays was positive, the patient was excluded from the study.

Cytokine determination: The protocol of cytokine measurement was reported in our previous study (13) as fol...
The clotted blood samples were centrifuged at 1,000 g, 20°C for 20 minutes. After that, the supernatant was collected and the levels of Th1/Th2 cytokines by 320 flow cytometry detected. IL-2, IL-4, IL-6, IL-10, TNF-α and IFN-γ were detected quantitatively by CBA kit-BD CBA Human Th1/Th2 Cytokine Kit II (BD Biosciences, San Jose, CA). After collecting the sample data on a FACScaniburTM flow cytometer (Becton Dickinson, San Jose, CA, USA), we used the BD CBA Software (BD Biosciences, San Jose, CA, USA) to display the results in tabular and graphical format. Then we established the standard curve for each reagent. 1.0 pg/mL was the lowest detection limit for these six cytokines, while the highest was 5,000 pg/mL.

Statistical analysis: We used χ² or Fisher’s exact test and Mann-Whitney U test in SPSS Statistics 19.0 software. It was considered that P < 0.05 is statistically significant. We used receiver operating characteristic (ROC) curve to estimate the value of cytokines in diagnosing M. pneumoniae pneumonia by SPSS Statistics 19.0 software.

4. Results

Patients’ characteristics: Between May 2012 and June 2014, 1,3161 throat swab samples were collected and tested for M. pneumoniae, including 1,353 from outpatients and 1,808 from hospitalized children. 8,082 samples were from boys and 5,079 from girls, yielding a male-to-female ratio of 1.59:1. 2188 were tested positive for M. pneumoniae, with a positive rate of 16.62%. Among 2188 M. pneumoniae positive samples, 1,277 were from boys and 911 from girls, giving positive rates of 15.80% in boys and 17.93% in girls.

As shown in Figure 1, positive rate for M. pneumoniae infection was at the peak in children aged 5-9 years (42.46% - 46.92%, compared with other group, P < 0.01). It steadily declined with increasing or decreasing age. Among age groups, children younger than 1 year had the lowest (7.39%) positive rate, compared with the other group (P < 0.01). M. pneumoniae infection occurred all year round, the monthly positive rates for M. pneumoniae infection ranged from 7.65% to 27.35%, with a peak in June and August (compared with other group P < 0.01), and steadily declined in the previous and the following months (Figure 2). Co-infections were found in 1,662 (75.96%) M. pneumoniae positive children, which were higher than in mono-infection children.

Inflammatory cytokine levels: To evaluate the levels of these six cytokines in healthy children and children with M. pneumoniae pneumonia. 526 patients with single M. pneumoniae infection were used as M. pneumoniae pneumonia group and 30 healthy children acted as the control group. As shown in Table 1, comparisons between M. pneumoniae infection group and normal control group revealed no difference of inflammatory cytokine (IL-6 and TNF-α) levels between the two groups (median levels, pg/mL: IL-6:14.27 vs. 4.54, P = 0.057; TNF-α: 3.56 vs. 2.21, P = 0.182). The level of IL-2 was significantly lower in serum from M. pneumoniae pneumonia patients than in serum from normal controls (median levels, pg/mL: IL-2: 3.19 vs. 5.72, P = 0.00), while the levels of IL-4, IL-10 and IFN-γ in M. pneumoniae pneumonia patients were significantly higher than in the normal controls (median levels, pg/mL: IL-4: 3.23 vs. 1.46, P = 0.00; IL-10: 5.56 vs. 2.53, P = 0.001; IFN-γ: 20.35 vs. 4.83, P = 0.001).

Figure 1. Age Distribution of M. Pneumoniae Pneumonia in Children Younger Than 14 Years

Figure 2. Monthly Distribution of M. Pneumoniae Pneumonia in Children Younger Than 14 Years.
ROC-analysis: To confirm value of inflammatory cytokines in diagnosis of *M. pneumoniae* pneumonia, we used ROC-analysis to evaluate the abilities of the six cytokines in identifying the possibility of *M. pneumoniae* pneumonia. The AUCs were 0.922 (95% CI, 0.878 to 0.966), 0.954 (95% CI, 0.928 to 0.979), 0.819 (95% CI, 0.758 to 0.880) and 0.928 (95% CI, 0.892 - 0.963) for IL-2 (lower than 4.5 pg/mL), IL-6 (greater than 2.5 pg/mL), IL-10 (greater than 3.0 pg/mL) and IFN-γ (greater than 5.5 pg/mL) respectively, with sensitivity and specificity above 80% (Table 2). These results indicated that IL-2, IL-4, IL-10 and IFN-γ could be effective biomarkers to identify *M. pneumoniae* pneumonia.

### Table 2. ROC Curve for Diagnostic Value of Cytokines for *Mycoplasma Pneumoniae* Pneumonia

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>AUC</th>
<th>95% CI</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
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<tbody>
<tr>
<td>IL-2</td>
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<td>.878 - .966</td>
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<tr>
<td>IL-4</td>
<td>.954</td>
<td>.928 - .979</td>
<td>81.9</td>
<td>100</td>
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<tr>
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<td>.678 - .780</td>
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<td>IL-10</td>
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<td>.758 - .880</td>
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<td>82.1</td>
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<tr>
<td>TNF-α</td>
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<td>.629 - .775</td>
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<td>78.8</td>
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<tr>
<td>IFN-γ</td>
<td>.928</td>
<td>.892 - .963</td>
<td>80.2</td>
<td>93.9</td>
</tr>
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</table>

References

7. Ratliff AE, Duffy LB, Waites KB. Comparison of the illumigene


