Cytokine 86 (2016) 73–78

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Serum cytokine profile contributes to discriminating *M. pneumoniae* pneumonia in children

Xue-Feng Xu, Xue-Jing Li, Jin-Ling Liu, Lei Wu, Zhi-Min Chen *

Department of Respiratory Medicine, The Children's Hospital, Zhejiang University School of Medicine, Hangzhou, China

ARTICLE INFO

Article history:
Received 27 February 2016
Received in revised form 14 July 2016
Accepted 24 July 2016
Available online 29 July 2016

Keywords:
Community-acquired pneumonia
Children
Cytokine
M. pneumoniae

ABSTRACT

Background: To evaluate the role of serum cytokines in discriminating *M. pneumoniae* infection in children with community-acquired pneumonia (CAP).
Methods: A prospective observational study was conducted. 385 hospitalized patients with CAP had only *M. pneumoniae* (MP group) infection; 321 hospitalized patients with CAP had no *M. pneumoniae* and other specific pathogen (control group) infections. Serum interleukin-2 (IL-2), IL-4, IL-6, IL-10, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were detected by flow cytometry.
Results: In children younger than 5 years, serum IL-6, TNF-α, and IFN-γ levels from MP group were significantly higher than those from control group. However in children 5–15 years, serum IL-6, IL-10, and IFN-γ levels from MP group were significantly higher than those from control group. In the final multivariate logistic regression model for serum cytokine, moderately elevated IL-6, IL-10, and IFN-γ shows a higher prediction of development of *M. pneumoniae* pneumonia among CAP patients.
Conclusions: A specific cytokine pattern showed a higher prediction of *M. pneumoniae* pneumonia among CAP patients, further suggesting that serum cytokine pattern might be useful in differentiating infectious causative agents in children.

1. Introduction

Community-acquired pneumonia (CAP) is the leading cause of death in children. Annually, >25% of children in the developing world will have an episode of pneumonia during the first 5 years of life and there are about 2 million deaths per year [1,2]. In addition to typical respiratory bacteria and viruses, *Mycoplasma pneumoniae* (*M. pneumoniae*) is a common causative agent of pneumonia in children [3]. Moreover, the causative agent of CAP differs according to the age of children. *M. pneumoniae* is the main pathogen causing CAP in children aged 5–15 years, while viruses are the predominant pathogen in preschool-aged children [4].

Although the clinical significance of *M. pneumoniae* infection is becoming evident, the underlying pathophysiological mechanisms contributing to the development of disease have not yet been fully understood. Recently, the role of cytokines in *M. pneumoniae* infections has gained much attention [5–9]. Cytokines are a group of small signal molecules secreted by activated lymphocytes, macrophages, and certain other cells, providing crucial functions in the host defenses to bacterial or viral infections. There is evidence indicated that cytokines such as interleukin-4 (IL-4), IL-5, IL-6, IL-10, and IFN-γ are closely associated with the development of *M. pneumoniae* infections or severity of disease either in patients or animal models [10–14].

Although we recognize the significant role of viruses in CAP in children, many children with viral infections may be receiving unnecessary antibiotics. In view of the difficulty of identifying specific pathogen in children with CAP, some inflammatory markers such as peripheral white blood cell (WBC) count, C-reactive protein (CRP), and procalcitonin (PCT), might play a role in distinguishing bacterial or non-bacterial (viruses) infections in patients with CAP [15–19]. However, these inflammatory markers showed to have a limited role, as it is a difficult to find a cutoff for any of these values that is both sensitive and specific in infectious agents. Similar to viral infection, most of *M. pneumoniae* pneumonia presented with normal or slightly increased WBC count and normal PCT value. A number of studies have shown that different cytokine profiles could be associated with various pathogens in adult CAP [20], and severity of disease [21–23]. However, the utility of cytokines in discriminating *M. pneumoniae* pneumonia in children was rarely reported.

In the present study, we evaluated the serum levels of cytokines to investigate their possible roles in the pathogenesis of acute *M. pneumoniae* pneumonia in children. A flow cytometry-based

* Corresponding author at: Department of Respiratory Medicine, The Children's Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China.
E-mail address: snowpeakxu@zju.edu.cn (Z.-M. Chen).

http://dx.doi.org/10.1016/j.cyto.2016.07.018
1043-4666/

inflammatory cytokine determination might be an effective and rapid diagnostic method to differentiate *M. pneumoniae* infection from those CAP patients with normal WBC counts and PCT value.

2. Methods

In the prospective observational cohort study, hospitalized children with CAP from the respiratory department of the Children's hospital of Zhejiang University School of Medicine between January 2013 and December 2014 were recruited. This study was approved by the Ethic Review Board of the Children's Hospital, Zhejiang University School of Medicine. Written informed consent was obtained from the parents of each child before enrollment. Those patients with tuberculosis or HIV positive were excluded from the study, those with bacterial infections or suspected bacterial infections were excluded, and those with chronic lung diseases, congenital heart disease, cerebral palsy or malignancy were also excluded. 385 patients with CAP had only *M. pneumoniae* infection, marked as MP group; 321 patients with CAP had no *M. pneumoniae* and other specific pathogen infections, labeled as control group. Meanwhile, those MP patients with specific concurrent viral infections were also excluded.

2.1. Definition of CAP

CAP was diagnosed by clinical symptoms (e.g. fever, cough), signs (e.g. chest rales), laboratory test (e.g. CRP, WBC count), chest radiographs. Diagnosis of *M. pneumoniae* pneumonia was based on diagnosis of CAP and etiology. Peripheral blood samples were taken for host response measurements: WBC count, serum CRP, and PCT. Respiratory immunofluorescence detections via nasopharyngeal aspirate (NPA) for respiratory syncytial virus, influenza virus A and B, parainfluenza virus, and adenovirus were performed. Serum on admission was collected for *M. pneumoniae* antibody measurements: IgM and IgG by means of an enzyme-linked immunosorbent assay (ELISA, Shanghai B&C Biological Technology, Co. Ltd., China) according to the manufacturer's instructions. The assay was considered positive if the IgM titer was higher than 1.1 [24]. *M. pneumoniae* DNA in NPA specimens or bronchoalveolar lavages (BAL) by bronchoscopy were detected by fluorescence quantitative real-time PCR according to "quantitative diagnostic kit for *M. pneumoniae* DNA" (PCR fluorescence probing) (Da An Gene Co., Ltd. of Sun Yat-sen University, China) based on the TaqMan PCR technology. Results with copy counts of *M. pneumoniae* DNA over 10^5 copies per 1 ml of specimen were regarded as positive [24]. Current infection with *M. pneumoniae* was based on $\geqslant$4-fold changes in antibody titers between paired acute and convalescent sera, positive IgM antibody, or positive *M. pneumoniae* DNA in NPA or BAL.

2.2. Cytokine measurement

Peripheral blood samples were collected at 24 h of admission. The blood sample was transferred to a serum separating tube and centrifuged at 1000*g* at room temperature for 20 min. The serum was carefully harvested and the measurement of the cytokines was performed by flow cytometry immediately; or the serum was temporarily stored at 2–8 °C until analysis (usually less than 12 h). Concentrations of IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ were quantitatively determined with the cytometric bead array (CBA) kit (CBA Human Th1/Th2 Cytokine Kit II; BD Biosciences, California) as described previously [25]. The minimal and maximum limits of detection for all cytokines were 1 pg/mL and 5000 pg/mL, respectively.

2.3. Statistics

Statistical analysis was performed using descriptive statistics such as frequency and percentage, mean and standard error of mean (SEM), or median. The continuous variables between groups were compared by Student's *t*-test. The logistic regression analysis was used to assess potential risk factors the development of *M. pneumoniae* pneumonia. The area under the receiver operating characteristic (ROC) curve is used to evaluate the predictive power of logistic regression model. The variables involved in the multivariate analysis included age, sex, fever duration, mode of delivery, birth weight, pleural effusion, and serum cytokine levels. All data were analyzed with PASW Statistics 18 software (SPSS Inc. Chicago, US). A p-value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Clinical characteristics

There was no difference in sex ratio among MP group and control group. The age of MP group children was higher than that of control group (5.07 ± 0.16 vs 2.89 ± 0.14 years, $P < 0.001$). The duration between the onset of clinical symptoms and admission in MP group was similar to that in control group (8.98 ± 0.21 vs 8.61 ± 0.31 days, $P = 0.324$); while the duration of fever on admission in MP group was significantly longer than that in control group (7.29 ± 0.20 vs 4.68 ± 0.28 days, $P < 0.001$). The counts of WBC in MP group were lower than those in control group (7.9 ± 0.18 vs 8.74 ± 0.25 × 10^9/L, $P = 0.006$), while neutrophil percentage (N%) in MP group was significantly higher than that in control group (62.97 ± 1.05% vs 49.42 ± 2.82%, $P < 0.001$). Although the values of PCT between two groups were less than 0.5 ng/L (normal value), the CRP value (reference value < 8 mg/L) in MP group was significantly higher than that in control group (31.03 ± 1.98 vs 13.77 ± 1.34, $P < 0.001$). According to CAP administration guideline in China's children by Pediatric Academy of Chinese Medical Association, beta-lactams are recommended for children <5 year, and macrolides are highly recommended for children >5 years. In the present study, there is no significant difference in antibiotics treatment between MP group and control group.

In children younger than 5 years, the duration of clinical symptoms on admission in MP group was similar to that in control group (9.13 ± 0.35 vs 8.71 ± 0.35 days, $P = 0.4$); while fever time on admission in MP group was longer than that in control group (6.62 ± 0.33 vs 4.80 ± 0.32, $P < 0.001$). The counts of WBC and PCT value in MP group were similar to those in control group ($P > 0.05$), while N% in MP group was significantly higher than that in control group (56.95 ± 1.92% vs 47.10 ± 3.44%, $P = 0.013$). Additionally, the value of CRP in MP group was significantly higher than that in control group (23.07 ± 2.54 vs 11.80 ± 1.40, $P < 0.001$). Compared with children younger than 5 years, the changes of duration of clinical symptoms, time of fever, counts of WBC (N%), PCT, and CRP among children 5–15 years between two groups also showed a similar trend.

3.2. Serum cytokine responses in relation to M. pneumoniae pneumonia

In children with CAP, no significant differences were observed in serum IL-2 and IL-4 levels between two groups (shown in Table 1). Serum IL-6 levels in MP group were significantly higher than those in control group (Table 1). The serum levels of TNF-α and IFN-γ were significantly higher in MP group patients compared to control group ($P < 0.01$). The increased IL-10 levels from MP group patients were similar to those from control group patients.