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Original article

Management of refractory *Mycoplasma pneumoniae* pneumonia: Utility of measuring serum lactate dehydrogenase level

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ABSTRACT

It has been suggested that cytokines are associated with refractory *Mycoplasma pneumoniae* pneumonia, and steroid administration is reported to be effective in this situation. In order to elucidate the characteristics of refractory *M. pneumoniae* pneumonia, we analyzed five pediatric patients with refractory *M. pneumoniae* pneumonia, which was defined as showing prolonged fever and deterioration of clinical and radiological findings despite administration of appropriate antibiotics, compared with 15 pediatric patients with *M. pneumoniae* pneumonia who responded to treatment promptly (control group). Serum lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and interleukin (IL)-18 levels were significantly higher in the refractory group than in the control group at the initiation of corticosteroid use (LDH: 571 vs 292 IU/L, $p = 0.0129$; ALT: 25 vs 11 IU/L, $p = 0.0143$; AST: 41 vs 26 IU/L, $p = 0.0404$; IL-18: 579 vs 365 pg/mL, $p = 0.0402$). Significant correlation was found between serum values of IL-18 and LDH ($r^2 = 0.504$, $p = 0.0433$). The administration of corticosteroids to patients in the refractory group resulted in the rapid improvement of symptoms and decrease in serum LDH levels in all patients. A serum LDH level of ≥410 IU/L, which was calculated from receiver operating characteristic curve analysis, seemed to be an appropriate criterion for the initiation of steroid therapy. In conclusion, serum IL-18 and LDH levels can be used as parameters to determine which patients are candidates for corticosteroid therapy. In addition, serum LDH levels seem to be a useful marker for the evaluation of therapeutic efficacy in refractory *M. pneumoniae* pneumonia.

1. Introduction

Mycoplasma pneumoniae is one of the major causative pathogens of community-acquired respiratory tract infections in children and younger adults. Although pneumonia due to *M. pneumoniae* is usually a benign, self-limited disease, some cases are known to develop into refractory or severe, life-threatening pneumonia despite the administration of appropriate antibiotics [1–7]. *M. pneumoniae* stimulates macrophages via toll-like receptors to release immunomodulatory and inflammatory cytokines and chemokines such as interleukin (IL)-18 and IL-8, which cause excessive immune responses resulting in the formation of pneumonia [1,8–11]. Corticosteroids down-regulate the cell-mediated immune response and, therefore, may have a profound effect by reducing the immune-mediated pulmonary injury seen in mycoplasmal infections [12]. Clinically, corticosteroids have been used for refractory *M. pneumoniae* pneumonia and are advocated for decreasing the inflammatory response with dramatic beneficial effect in both children and adults [2–7]. Thus, it is desirable to investigate appropriate conditions for the initiation of steroid treatment. In this study, we analyzed the clinical findings of pediatric patients with refractory *M. pneumoniae* pneumonia, which was defined as showing prolonged fever and deterioration of clinical and radiological findings despite administration of appropriate

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antibiotics, compared with pediatric patients with *M. pneumoniae* pneumonia who responded to treatment promptly.

2. Patients and methods

2.1. Patient characteristics

Twenty pediatric patients with *M. pneumoniae* pneumonia admitted Kawasaki Medical School Hospital and Yamaguchi University Hospital from April 2010 to November 2012 were enrolled in this study. *M. pneumoniae* pneumonia was diagnosed by a positive polymerase chain reaction (PCR) result or four-fold increase in antibody titer, as reported previously [13,14]. *M. pneumonia* DNA was detected by real-time PCR targeting a conserved part of the gene encoding P1 adhesin. Antibodies to *M. pneumoniae* were measured using a particle agglutination test (Serodia-Myco II kit, Fujirebio, Tokyo, Japan). Of the 20 cases, eight cases were PCR positive and all cases were positive by serology. Informed consent was obtained from all patients, and the study protocol was approved by the Ethics Committee at Kawasaki Medical School.

The severity of pneumonia was assessed by the use of a clinical severity scale published in the guidelines for the management of respiratory infectious diseases in children in Japan, which was evaluated from data regarding physical examination findings, chest X-ray findings, and laboratory data [15].

2.2. Laboratory tests

Blood tests for peripheral white blood cell (WBC) count, C-reactive protein (CRP), total protein, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and various cytokines were performed on admission, at the initiation of steroid use, and at the end of treatment. The serum levels of IL-2, IL-4, IL-6, IL-10, interferon γ (IFN-γ), and tumor necrosis factor α (TNF-α) were determined using a cytometric bead array kit (BD PharMingen, San Diego, CA, USA), and that of active form IL-18 was determined using a Human IL-18 ELISA kit (MBL Co. Ltd, Japan).

2.3. Detection of point mutations for macrolide resistance in domain V of 23S rRNA

A search for mutations at sites 2063, 2064, and 2617 in the *M. pneumoniae* 23S rRNA domain V gene region was performed using a direct sequencing method in samples with a positive PCR result, as reported previously [13,14]. Five of eight PCR positive cases were found to be infected with macrolide-resistant (MR) *M. pneumoniae*. Among these five patients with MR *M. pneumoniae*; all had an A-to-G transition at position 2063 in domain V of the 23S rRNA gene (A2063G).

2.4. Statistical analysis

Statistical analysis was performed using Stat View version 5.0. (SAS Institute Inc, Cary, NC, USA). The incidence of underlying conditions and pneumonia severity were analyzed using Fisher's exact test. Mean age of patients were compared using Student's *t* test and laboratory data were compared using Mann–Whitney's *U* test. To set the cut-off level of laboratory data for the initiation of steroid therapy was calculated employing a receiver operating characteristic (ROC) curve [16]. The laboratory data were used at the admission in 15 patients with non-refractory group and at the initiation of steroid use in 5 patients with refractory group.

3. Results

3.1. Patient characteristics

Categorical variables age, gender, co-morbid illness, pneumonia severity, mutation type, prior prescription of antibiotics before visiting the study clinic or hospital, and effective antibiotics of 20 pediatric patients with *M. pneumoniae* pneumonia are presented in Table 1. Of these, five cases were refractory, which was defined as showing prolonged fever and deterioration of clinical and radiological findings after administration of appropriate antibiotics for 7 days or more. After admission to hospital, all patients were administered oxygen for hypoxemia. Among patients in the refractory group, three patients had MR *M. pneumoniae* pneumonia but they received minocycline or tosufloxacin, which are effective against MR strains [13,14].

3.2. Laboratory findings

The laboratory findings of the two groups are presented in Table 2. At admission, no significant differences were observed between the refractory group and the control group. At the initiation of steroid use, serum LDH, ALT, AST and IL-18 levels were significantly higher in the refractory group than in the control group (LDH: 571 vs 292 IU/L, $p = 0.0129$; ALT: 25 vs 11 IU/L, $p = 0.0143$; AST: 41 vs 26 IU/L, $p = 0.0404$; IL-18: 579 vs 365 pg/mL, $p = 0.0402$).

3.3. Relationship between IL-18 levels and other clinical laboratory data

The association between serum IL-18 values and other clinical laboratory data was evaluated with the Spearman's rank correlation coefficient. The laboratory data were used at the admission in 15 patients with non-refractory group and at the initiation of

Table 1
Categorical variables age, gender, co-morbid illness, pneumonia severity, mutation type, prior prescription of antibiotics before visiting the study clinic or hospital, and effective antibiotics of 20 pediatric patients with *M. pneumoniae* pneumonia.

Variables	Refractory group	Control group	*p*-Value
No. of patients	5	15	
Mean age (range), years	6.2 ± 1.72 (4–9)	8.0 ± 3.56 (1–14)	0.315
No. of males/females	3/2	8/7	0.795
No. of patients with co-morbid illness	0	4	0.530
Pneumonia severity			
Mild	0	8	0.040
Moderate	4	7	
Severe	1	0	
No. of resistant cases/*M. pneumoniae* PCR positive cases	3/4	2/4	
No. of point mutation in domain V of 23S rRNA	A2063G 3	A2063G 2	
No. (%) of patients with prior prescription			
Azithromycin	4	2	
Cefditoren pivoxil	0	1	
No. (%) of patients with effective antibiotics			
Minocycline	4 (+steroid)	4	
Quinolones	1 (+steroid)[a]	4[b]	
Clarithromycin	0	3	
Clindamycin	0	1	
Cefditoren pivoxil	0	3	

[a] Patient received tosufloxacin.
[b] Two patients received tosufloxacin, one received levofloxacin and one received norfloxacin.