

Atopy: a risk factor of refractory mycoplasma pneumoniae pneumonia?

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Abstract

Objective: To investigate the relationship of pathogen DNA copies with clinic and laboratory features among children with *Mycoplasma pneumoniae* (MP) pneumonia.

Methods: A total of 95 enrolled children with MP pneumonia were assigned into the high-MP-load group ($>10^6$ /mL) and the low-MP-load group ($\leq 10^6$ /mL) according to MP-DNA copies in bronchoalveolar lavage fluid (BALF). Clinical characteristics and any allergy history were collected. Aeroallergens and food allergens were detected with a skin test. Serum IgE and eosinophil cationic protein (ECP) were assessed using enzyme immunoassay. BALF levels of IL-4, IFN- γ , IL-8 and TNF- α were assessed by ELISA.

Results: Compared with the low-MP-load group, 72.7% in the high-MP-load group developed refractory MP pneumonia who failed to respond to at least 1-week treatment with macrolides (72.7% vs 41.9%, $P = 0.005$). More children in the high-load group than those in the low-load group presented with extrapulmonary manifestations, lung consolidation, pleural effusion and atopic conditions including any allergy history, positive findings of aeroallergen test and increased serum IgE and ECP ($P < 0.05$). A significant higher BALF IL-4 level was seen in the high-load group versus the low-load group (23.00 ± 11.24 vs 14.68 ± 7.12 ; pg/mL; $P < 0.01$). There were no significant differences in BALF levels of IFN- γ , IL-8 and TNF- α between the two groups ($P > 0.05$).

Conclusion: Atopy may be a risk factor for the presence and severity of refractory MP pneumonia due to the high pathogen load in airway.

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Ethics

The study was approved by the institutional review committee and was conducted according to the Declaration of Helsinki and all subsequent revisions.

Conflict of interest

The authors declare that they have no conflicts of interest.

Key words

atopy – bronchoalveolar lavage fluid – DNA copy numbers – mycoplasma pneumoniae – refractory mycoplasma pneumoniae pneumonia

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Authorship and contributorship

Dr. YX Bao performed the clinical studies and statistical analysis and drafted the manuscript. Dr. Jing Li collected the data and performed fiber bronchoscopy. Drs. Y Tian and QH Liu performed enzyme-linked immunosorbent assay and real-time quantitative polymerase chain reaction. Dr. J Bao conceived the study, participated in its design and coordination and revised the manuscript. All of the authors read and approved the final version of the manuscript.

Abbreviations

BALF bronchoalveolar lavage fluid
ECP eosinophil cationic protein
EIA enzyme immunoassay
ELISA enzyme-linked immunosorbent assay
IFN interferon

Ig immunoglobulin
IL interleukin
MP *Mycoplasma pneumoniae*
RT-PCR real-time quantitative polymerase chain reaction
TNF tumor necrosis factor

Introduction

Mycoplasma pneumoniae (MP) associated pneumonia occurs frequently in children and may be effectively treated with macrolides. A 'refractory' case presents with clinical and radiographic progression despite appropriate antibiotic therapy for 7 days or more, as defined as Tamura A *et al.* (1). In recent years, an increasing number of severe and refractory cases have been reported. However, the pathogenesis is still unclear yet (1, 2). Some epidemiological investigations revealed that severe low respiratory tract infections were associated with the presence of underlying medical conditions such as asthma (3, 4). Shin JE *et al.* (5) found that children with an allergic disease were more likely to develop progressive MP pneumonia despite at least 5 days of macrolide antibiotic treatment. No further data have been available to indicate the relationship between atopy and refractory MP pneumonia. In this study, we investigated clinic features including atopic conditions, bronchoalveolar lavage fluid (BALF) expressions of cytokines and MP DNA copy numbers among children with MP pneumonia, to find out some risk factors for refractory cases.

Methods

Patients

Eligible children were 1–13 years old and diagnosed as MP pneumonia on the basis of radiologic findings of lobar or bronchopneumonia and serum antimycoplasma antibody titer of 1:160 or greater (immunofluorescence kits, Euroimmun, Germany). Refractory MP pneumonia was defined as clinical and radiographic progression despite at least 7-days macrolide antibiotic treatment (1). No bacteria or viruses were detected in the sputum and blood tests. All of the subjects' legal guardian and subjects aged 10 years and older provided written informed consent. The study protocol was approved by the institutional review committee and was conducted according to the Declaration of Helsinki and all subsequent revisions.

Study protocol

A total of 95 hospitalised children with MP pneumonia were enrolled into the study at Shanghai Xinhua Hospital of China between January and December 2013. Patient characteristics and any allergy history were recorded including age, sex, duration of fever, extrapulmonary manifestations such as autoimmune responses, central nervous system complications and dermatological disorders, chest radiographic findings of consolidation or pleural effusion, and a history of any

atopic condition including eczema, urticaria, allergic rhinitis, asthma, allergies to medications or foods and any other allergic diseases. Aeroallergens and food allergens were detected with a skin prick test. Serum levels of total immunoglobulin (Ig) E and eosinophil cationic protein (ECP) were assessed using fluorescence enzyme immunoassay (EIA) (ImmunoCAP Systems, Pharmacia).

All the enrolled patients were undergone fiberoptic bronchoscopy to collect BALF. Following centrifugation at 3000 r/min for 10 min, the supernatant BALF was used to assess levels of interferon (IFN) γ , interleukin (IL) 4, IL-8 and tumor necrosis factor (TNF) α by enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, USA). The cell deposits from BALF were used to extract DNA and determine the copy number of MP 16s rRNA by real-time quantitative polymerase chain reaction (RT-PCR) (StepOnePlus, Applied Biosystems, USA). According to MP-DNA copies, all the subjects were assigned into the high-MP-load group ($>10^6$ /mL) and the low-MP-load group ($\leq 10^6$ /mL).

Statistical analysis

The categorical data, such as proportion of refractory MP pneumonia, were analysed using chi-square test measures. The continuous variables and BALF expressions of cytokines were analysed using Student's paired *t*-test (parametric) or Wilcoxon's rank-sum test (non-parametric) when appropriate. Two-tailed *P* values of 0.05 or less were considered statistically significant.

Results

Patient characteristics and MP load

Of the enrolled 95 children with MP pneumonia, 42 were male and 53 female. The ages ranged from 1 to 13 (5.8 ± 2.62) years old. Fifty (52.6%) children met with the definition of 'refractory' cases. Thirty (31.6%) children had at least one history of allergic disease. Positive findings were seen in 46 (48.4%) children with aeroallergen test, 30 (31.6%) children with food allergen test. Thirty-nine (41.1%) children had an increased total IgE level (normal <100 IU/mL). Sixty-four (67.4%) children had an increased serum ECP level (normal <8 μ g/L).

According to the assessed MP-DNA copies, 33 (34.7%) subjects were assigned into the high-MP-load group ($>10^6$ /mL) and the other 62 (65.3%) were the low-MP-load group ($\leq 10^6$ /mL). Twenty-four (72.7%) subjects in the high-MP-load group developed refractory MP pneumonia compared with the low-load group (72.7% vs 41.9%, $P = 0.005$). The high-MP-load group had a markedly longer duration of fever than

Table 1. Relationship of pathogen load with clinical and laboratory characteristics in 95 children with MP pneumonia

	High MP load (n = 33)	Low MP load (n = 62)	P value
Male, n (%)	12(36.4)	30(48.4)	0.286
Age <6 years old, n (%)	15(45.5)	27(43.5)	1.000
Refractory MP pneumonia, n (%)	24(72.7)	26(41.9)	0.005
Duration of fever (day)	8.40 ± 2.58	6.35 ± 2.54	0.000
Extrapulmonary manifestations, n (%)	9(27.3)	0(0)	0.000
Consolidation on chest X-ray, n (%)	21(63.6)	25(40.3)	0.034
Pleural effusion, n (%)	10(30.3)	7(11.3)	0.027
Atopy			
Any allergic histories, n (%)	18(54.5)	12(19.4)	0.000
Positive aeroallergen test, n (%)	21(63.6)	25(40.3)	0.034
Positive food allergen test, n (%)	9(27.3)	21(33.9)	0.644
Total IgE ≥100 IU/mL, n (%)	24(72.7)	15(24.2)	0.000
ECP ≥8 µg/L, n (%)	27(81.8)	37(59.6)	0.038
BALF cytokines			
IFN-γ (pg/mL)	31.55 ± 14.82	27.54 ± 9.80	0.124
IL-8 (pg/mL)	117.24 ± 53.41	114.48 ± 67.49	0.627
TNF-α (pg/mL)	117.57 ± 51.41	101.98 ± 42.50	0.122
IL-4 (pg/mL)	23.00 ± 11.24	14.68 ± 7.12	0.000

Quantitative values were expressed as mean ± SD. BALF, bronchoalveolar lavage fluid; ECP, eosinophil cationic protein; IFN, interferon; Ig, immunoglobulin; IL, interleukin; MP, Mycoplasma pneumoniae; TNF, tumor necrosis factor.

the low-MP-load group (8.40 ± 2.58 vs 6.35 ± 2.54 day, $P = 0.000$). Significantly greater proportions of extrapulmonary manifestations, lung consolidation and pleural effusion were also observed in the high-MP-load group compared with the low-load group ($P < 0.05$, Table 1).

Atopy and MP load

Compared with the low-MP-load group, a significantly more children in the high-load group had at least one history of allergic disease, positive findings of aeroallergen test, increased serum levels in total IgE and ECP ($P < 0.05$, Table 1). No significant difference was seen in the positive rate of food allergen test between the two groups ($P > 0.05$, Table 1).

BALF IFN-γ, TNF-α, IL-8 and IL-4 and MP load

The high-MP-load group had a significantly higher IL-4 level in BALF than the low-MP-load group (23.00 ± 11.24 vs 14.68 ± 7.12 , pg/mL; $P = 0.000$). There were no significant differences in BALF levels of IFN-γ, TNF-α and IL-8 between the two groups ($P > 0.05$, Table 1).

Discussion

In our study, 52.6% of the 95 hospitalised children with MP pneumonia were refractory to at least 1-week treatment of macrolides. The reasons for refractory MP pneumonia have not been elucidated. Miyashita et al.

(7) indicated that persistent fever despite 48 h of macrolides use may be suggestive of macrolide resistance, which is now highly prevalent in East Asian countries, especially in China (8, 9). Our results showed that more children in the high-MP-load group were refractory to at least one-week treatment with macrolide, confirming that refractory MP pneumonia associated with high pathogen load may be due to macrolide resistance. It has been shown that the severity of MP infection may be related to pathogen load in airway secretions (10). Compared with the low-load group, the high-load group in this study had a longer duration of fever and were more likely to present with extrapulmonary manifestations, lung consolidation and pleural effusion. The results indicate the need of effective macrolides antibiotic treatment to quickly reduce MP load and improve clinical outcomes.

Besides, we found that MP load was positively related to atopic conditions including history of allergic diseases, positive findings of aeroallergen test and increased serum levels in total IgE and ECP, as well as BALF IL-4 level. IL-4, a Th2-producing cytokine, is a key regulator in the development of allergies by inducing B-cell class switching to IgE (11). Mice deficient for T-bet are used as animal model of asthma, which may produce less IFN-γ and more IL-4. Bakshi CS et al.'s animal research revealed that T-bet deficiency facilitated MP colonization in airway due to the lack of IFN-γ production and the failure of IL-4 to clear MP (12). These findings may partly explain the

relationship between the high MP load and atopic conditions and indicate that atopy may be a high risk factor for refractory MP infection due to high pathogen load.

Cytokines plays an important role in the lung inflammation. However, it is still under debate whether proinflammatory cytokines are involved in the development refractory MP pneumonia (13). Matsuda K *et al.* (14) reported that patients with macrolide-resistant MP infection had higher serum IFN- γ and IL-6 levels. In contrast, we also investigated BALF levels of cytokines but found no relationship of MP load with IFN- γ , TNF- α and IL-8.

In conclusion, the presence and severity of refractory MP pneumonia is associated with high MP load. Atopy may be a risk factor of refractory MP pneumonia due to the high pathogen load in airway.

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