Observational Study

Medicine®

OPEN

Impact of Epstein-Barr virus coinfection in *Mycoplasma pneumoniae* pneumonia

Yingchun Xu, MD[a], Shuxian Li, MD[a], Jinling Liu, MD[a], Junfen Zhou, MD[a,b], Fang Jin, MD[a], Xiaoyang Chen, MD[c], Yingshuo Wang, MD[a], Yuan Jiang, MD[a], Zhimin Chen, MD, PhD[a,*]

Abstract

Mycoplasma pneumoniae (MP) is one of the most common pathogens of respiratory infection in children, while Epstein-Barr virus (EBV) infection is usually subclinical in immunocompetent children. Although single MP infection is common enough, MP and EBV coinfection have received little attention. Especially, the pathogenic role of EBV in lung when coinfection with MP, has not been clarified. The purpose of this study was to investigate the impact of EBV on MP pneumonia (MPP) in hospitalized children. We retrospectively reviewed the clinical data of MPP children who underwent screening for EBV by polymerase chain reaction in bronchoalveolar lavage fluid during hospitalization in 2014. Of total 147 patients, 68 patients were in the MP group and 79 were in the MP/EBV coinfection group. We found longer fever duration and higher CRP, IgA, IgG, interleukin-2 (IL-2), percentage of peripheral neutrophils levels, higher incidence of pulmonary consolidation and percentage of refractory MPP in coinfection group, when compared to those in MP group. In ROC curve analysis, IL-2 was useful for differentiating patients with coinfection from those with MP infection. Logistic regression analysis showed that the IL-2 ≥ 3.35 pg/ml (OR = 3.677) was a significant predictor regarding to MP/EBV coinfection. In conclusion, coinfection of EBV and MP poses a higher risk for prolonged symptoms. IL-2 could be used as a good predictor of coinfection.

Abbreviations: BALF = bronchoalveolar lavage fluid, CAP = community acquired pneumonia, EBV = Epstein-Barr virus, IL-2 = interleukin-2, MP = *Mycoplasma pneumoniae*, MPP = *Mycoplasma pneumoniae* pneumonia, RMPP = refractory *Mycoplasma pneumoniae* pneumonia, ROC = receiver operating characteristics.

Keywords: coinfection, Epstein-Barr virus, interleukin-2, mycoplasma pneumonia

1. Introduction

Mycoplasma pneumoniae (MP) as one of the most frequent causes of community acquired pneumonia (CAP),[1] accounts for up to 40% of the CAP cases in children.[2] Previous studies showed that 18.5% to 30% of hospitalized children with CAP had evidence of concomitant viral-bacterial infection.[3–6] Coinfection of MP is common in children with CAP.[6–8] For instance, Chen et al demonstrated a high incidence (37%) of MP infection in Taiwanese children with CAP, among which 59.7% were coinfected with other pathogens, including *Streptococcus pneumoniae*, chlamydia, respiratory syncytial viral (RSV), influenza A, parainfluenza, adenovirus (ADV).[7] Similarly, MP and virus coinfection were also identified in a multicenter study conducted by Hao et al.[6] Although MP pneumonia (MPP) is generally a benign self-limited disease, some cases may become refractory. Besides excessive immune responses, coinfection is also associated with refractory MPP (RMPP).[9] Thus, the study of coinfection of MPP might explore potential benefits of the early use of appropriate drugs for RMPP.

The known causative viral pathogens in childhood CAP are mainly RSV, ADV, influenza A and B viruses (IVA and IVB), parainfluenza viruses 1–3 (PIVs 1–3) and human rhinovirus (HRV).[10,11] With regard to virus infections, Epstein-Barr virus (EBV) infection has traditionally been paid little attention, except in infectious mononucleosis (IM) in immunocompetent patients and lymphoproliferative disorders in immunocompromised hosts. EBV as a member of the herpesvirus family, its infection is usually asymptomatic.[12] It is reported that mild, asymptomatic pneumonia was found in about 5% to 10% of cases of EBV infection.[13] However, its pathogenic role in respiratory tract infections in immunocompetent patients remains poorly understood. Moreover, coinfection of EBV and MP in lung in immunocompetent children have received little attention and rarely been reported. Therefore, the aim of this study was to gain a better understanding of the clinical and pathological signifi-

Editor: Oliver Schildgen.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

YX and SL contributed equally to this work.

The work was supported by grants from the National Nature Science Foundation of China (No. 81801552), the Education Department Project of Zhejiang Province (No. Y201534178), and the Zhejiang Provincial Natural Science Foundation (Nos. LQ14H010001 and LQ20H190006).

The authors report no conflicts of interest.

[a] Department of Pulmonology, The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, Hangzhou, [b] Department of Pediatrics, Wenling Maternal and Child Health Care Hospital, Wenling, [c] Department of Developmental and Behavioral, The Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, Hangzhou, Zhejiang, China.

[*] Correspondence: Zhimin Chen, Department of Pulmonology, The Children's Hospital, Zhejiang University School of Medicine, 3333 Binsheng Road, Hangzhou, Zhejiang 310052, China (e-mail: zmchen@zju.edu.cn).

How to cite this article: Xu Y, Li S, Liu J, Zhou J, Jin F, Chen X, Wang Y, Jiang Y, Chen Z. Impact of Epstein-Barr virus coinfection in Mycoplasma pneumoniae pneumonia. Medicine 2020;99:16(e19792).

Received: 21 December 2019 / Received in final form: 26 February 2020 / Accepted: 27 February 2020

http://dx.doi.org/10.1097/MD.0000000000019792

cance EBV coinfection on MPP in hospitalized children. The clinical and laboratory characteristics in such instances were also defined and discussed.

2. Materials and methods

2.1. Study population

We retrospectively collected the clinical data of patients with pneumonia who were treated with fiber optic bronchoscopy (FOB) in Children's hospital, Zhejiang University School of Medicine between January 1, 2014 and December 31, 2014.

The inclusion criteria for our study included

(1) patients with signs and symptoms indicative of pneumonia on admission, including fever, cough, abnormal lung auscultation and a new infiltrate on chest radiograph;
(2) The diagnosis of MP infection was confirmed by the positive results for MP polymerase chain reaction (PCR) tests of bronchoalveolar lavage fluid (BALF).

Likewise, EBV infection was based on the positive results of EBV PCR of BALF. All patients with large pulmonary lesions or lung inflammation difficult to absorb were treated with alveolar irrigation and drainage using FOB.[14] MPP patients showing clinical and radiological deterioration despite of macrolide antibiotic therapy for 7 days or longer was defined as RMPP.[15]

The exclusion criteria for our study were

(1) patients with immune deficiencies, chronic diseases, heart diseases, neurological disorders, bronchopulmonary dysplasia, metabolic diseases, congenital disease and who were using immunosuppressive drugs;
(2) besides MP and EBV, those coinfected with other pathogens; and
(3) those with incomplete clinical data.

This study was conducted with the approval of the Ethics Committee of the Children's Hospital, Zhejiang University School of Medicine. All methods were conducted in accordance with the Declaration of Helsinki. Written informed consent was received from legal guardians of each patient.

2.2. Data collection

Data regarding demographic, clinical information, laboratory data, radiological and FOB findings from all enrolled patients were retrospectively collected. Laboratory specimens were obtained including blood, nasopharyngeal aspirates (NPAs), and BALF. Peripheral blood samples were obtained on admission for the determination of white blood cell count, neutrophils % (N%), platelet (PLT) count, C-reactive protein (CRP), humoral immunity (including IgA, IgG, IgM and IgE), cell-mediated immunity (including CD3+, CD4+ and CD8+ cells), cytokines (including interleukin-2 [IL-2], IL-4, IL-6, IL-10, interferon γ [IFN-γ] and tumor necrosis factor α [TNF-α]) and serology for Chlamydia pneumonia (CP), Chlamydia trachomatis (CT), Legionella pneumophila (LG) and EBV (including capsid antigen [VCA], early antigen [EA] and nuclear antigen [NA]). To rule out other viruses (including RSV, influenza viruses, metapneumovirus, adenovirus, and parainfluenza virus) or bacterial coinfection, NPAs were obtained during the first 24 hours of hospitalization for virus antigens detection and bacterial culture.

2.3. BALF collection and MP/EBV gene detection

The procedure of BALF collection was performed as described previously.[14] Briefly, BALF samples were collected for MP and EBV DNA detection, and the remaining samples were counted after centrifuged at $200 \times g$ for 10 minutes at 4°C. MP or EBV DNA was detected on a 7500 Real-time PCR System (Applied Biosystems) using MP PCR kit (Daan Gene Ltd Co., Guangzhou, China) or EBV PCR kit (Daan Gene Ltd Co., Guangzhou, China) based on the TaqMan PCR technology.

2.4. Measurement of serum cytokines

The serum concentrations of IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α were determined using a cytometric bead array (CBA) Human Th1/Th2 Cytokine kit II (BD Biosciences, San Diego, CA) according to the manufacturer's specifications as described previously.[16]

2.5. Statistical analysis

Statistical analysis was performed using SPSS 23.0. Data were reported as numbers with percentages for categorical variables, and as mean ± standard deviation (SD) or median with interquartile range (IQR) for continuous variables. Meanwhile, $\chi 2$ test or fisher exact test for categorical data, and parametric or nonparametric comparative tests for continuous data were used to compare variables between groups. Area under the receiver operating characteristics (ROC) curve was to evaluate candidate indicators with regard to the assessment of patients with coinfection. Logistic regression analysis was performed to select the variables associated with the coinfection. $P < .05$ were considered statistically significant.

3. Results

3.1. Clinical characteristics

A total of 147 patients were finally recruited into this study, of which the MP/EBV coinfection group accounted for 79 (53.7%) cases and MP group for 68 (46.3%) cases. None of the patients with MP/EBV coinfection had a history of infectious mononucleosis. Relevant demographic, clinical and laboratory data of these patients were shown in Table 1. In brief, no difference was found in age, gender distribution, and duration of hospitalization between these 2 groups. However, coinfection group had a longer duration of fever compared with MP infection group. As some patients displayed clinical and radiological progression after macrolide therapy for 7 days or longer, 32 patients were defined as RMPP in MP group, while 51 patients in coinfection group ($P = .033$).

Regarding the laboratory examinations, the median levels of CRP, IgA, IgG and the median percentage of peripheral neutrophils in coinfection group were significant higher than those in MP group, although no significant differences were observed in the median values of WBC, ALT, AST, LDH, CK-MB, IgE, IgM, and subtypes of T lymphocytes between the 2 groups.

Furthermore, the EBV DNA copies in BALF in coinfection group range from 1.0×10^3 to 1.8×10^8/ml. However, there was no difference in BALF MP DNA copies and neutrophil percentages between the 2 groups.

Table 1

Baseline demographics of the patients.

Characteristic	MP (n = 68)	MP/EBV (n = 79)	P
Age (years)	5.08 ± 3.16	5.79 ± 2.97	.161
Sex (male/female)	41/27	46/33	.799
Hospital stays (days)	8.0 (6.0–11.0)	8.0 (6.0–12.0)	.617
Total fever duration (days)	11.0 (8.0–13.8)	13.0 (10.0–15.0)	.039
Laboratory examinations			
WBC ($\times 10^9$/L)	7.45 (5.45–11.15)	8.40 (6.61–10.66)	.385
Neutrophil (%)	63.10 (45.45–73.98)	68.40 (59.40–75.70)	.023
C-reactive protein (CRP, mg/L)	20.50 (6.00–56.25)	34.00 (15.00–75.00)	.040
Alanine transaminase (ALT, U/L)	16.5 (13.0–27.8)	15.0 (9.0–25.0)	.098
Aspartate aminotransferase (AST, U/L)	36.0 (25.0–46.8)	31.0 (24.0–50.0)	.511
Lactate dehydrogenase (LDH, U/L)	391.0 (301.8–602.75)	404.0 (281.0–545.0)	.787
Creatine kinase isoenzyme (CK-MB, U/L)	14.0 (11.0–19.75)	14.0 (11.0–22.0)	.738
BALF			
EBV-DNA (copy/ml)	–	1.42×10^4 (3.6×10^3–8.6×10^4)	–
EBV-DNA (log copy/ml)	–	4.15 (3.56–4.93)	–
MP-DNA (copy/ml)	1.5×10^7 (5.45×10^5–7.63×10^7)	1.2×10^7 (1.83×10^6–3.9×10^7)	.621
MP-DNA (log copy/ml)	7.17 (5.73–7.88)	7.08 (6.26–7.59)	.621
Neutrophil (%)	38.00 (25.00–83.75)	35.00 (22.00–71.00)	.282
Cell-mediated immunity			
CD3 + cells (%)	62.48 (53.14–69.93)	62.84 (53.79–70.04)	.817
CD4 + cells (%)	32.04 ± 10.02	32.68 ± 10.26	.690
CD8 + cells (%)	21.81 (17.77–29.93)	21.97 (18.43–25.98)	.454
Humoral immunity			
IgE (g/L)	107.00 (32.40–215.00)	68.90 (29.90–170.50)	.362
IgA (g/L)	0.94 (0.48–1.35)	1.25 (0.90–1.57)	.008
IgM (g/L)	1.13 (0.72–1.69)	1.19 (0.75–1.44)	.979
IgG (g/L)	8.00 (6.92–9.80)	9.60 (7.76–11.20)	.024

EBV = Epstein-Barr virus.

Table 2

Types of antibody response against EBV in patients with MP/EBV coinfection.

	Antibodies against EBV antigens					
Types of antibody response	VCA-IgM	VCA-IgG	EA-IgM	EA-IgG	NA-IgG	n (percentage)
1	–	+	–	–	–	7 (8.9%)
2	–	+	–	–	+	46 (58.2%)
3	–	+	+	–	–	3 (3.8%)
4	+	+	–	–	+	5 (6.3%)
5	–	+	–	+	+	11 (13.9%)
6	–	+	+	–	+	3 (3.8%)
7	+	+	–	+	+	1 (1.3%)
8	–	+	+	+	+	3 (3.8%)

MP = *Mycoplasma pneumoniae*, EBV = Epstein-Barr virus, VCA = EBV capsid antigen, EA = EBV early antigen, NA = EBV nuclear antigen.

Of note, there were 8 different types of antibody response against EBV in coinfection group (Table 2). The positive rate of VCA-IgM, VCA-IgG, EA-IgM, EA-IgG, and NA-IgG in serum of coinfection patients were 7.6%, 100.0%, 11.4%, 19.0%, and 87.3%, respectively.

In addition to laboratory data, radiological findings showed severe pulmonary complications in both groups (Table 3). In detail, there were significant differences between the groups in the incidence of pulmonary consolidation (8.8% vs 29.1%, $P = .002$). However, the difference in the incidence of lobar atelectasis, pleural effusion, mediastinal emphesema and necrotizing pneumonia did not reach statistical significance.

Of the 147 patients, extra-pulmonary complications were found in 38 cases (25.9%). In detail, the patient number of extra-pulmonary complications was 17 (25.0%) in MP group and 21 (26.6%) in coinfection group, without a significant difference ($P = .827$). Among patients with extra-pulmonary complications of MP group, only 1 subject had 2 extra-pulmonary systems involvement and the remaining patients had only 1 extra-pulmonary system involvement. In the coinfection group, only 1 extra-pulmonary system involvement was observed in 15

Table 3

Radiological features of the patients.

Radiological features	MP (n = 68)	MP/EBV (n = 79)	P
Pulmonary consolidation	6 (8.8%)	23 (29.1%)	.002
Lobar atelectasis	21 (30.9%)	21 (26.6%)	.565
Pleural effusion	22 (32.4%)	30 (38.0%)	.477
Mediastinal emphesema	0 (0.0%)	2 (2.5%)	.499
Necrotizing pneumonia	2 (2.9%)	1 (1.3%)	.596

EBV = Epstein-Barr virus; MP = Mycoplasma pneumoniae.

Table 4

Extra-pulmonary complications of the patients.

Extra-pulmonary complications	MP (n = 68)	MP/EBV (n = 79)	*P*
Liver dysfunction	7 (10.3%)	9 (11.4%)	.831
Myocardial injury	10 (14.7%)	14 (17.7%)	.622
Granulocytopenia	0 (0.0%)	2 (2.5%)	.499
Thrombocytopenia	0 (0.0%)	1 (1.3%)	1.000
Hypokalemia	1 (1.5%)	2 (2.5%)	1.000
Rash	0 (0.0%)	1 (1.3%)	1.000

EBV = Epstein-Barr virus; MP = Mycoplasma pneumoniae.

patients, while 2 and more than systems involvement were observed in 5 patients and 1 patient, respectively. In total, extra-pulmonary complications including liver dysfunction, myocardial injury, granulocytopenia, thrombocytopenia, hypokalemia, and rash involvement were seen in 16 (10.9%), 24 (16.3%), 2 (1.4%), 1 (0.7%), 3 (2.0%), and 1 (0.7%) case, respectively (Table 4). Unfortunately, there was no significant differences between the 2 groups in the incidence of liver dysfunction, myocardial injury, granulocytopenia, thrombocytopenia, hypokalemia, and rash (Table 4).

3.2. Comparison of the serum cytokines between the MP group and the coinfection group

A comparison of the serum cytokines between the MP group and the coinfection group was presented in Figure 1. In detail, the serum IL-2 concentration was significantly higher in coinfection group (3.50 (2.50–4.50) pg/ml) than those in MP group (2.75 (1.93–3.70) pg/ml) (Fig. 1A). However, serum IL-4 (3.20 (2.80–4.10) pg/ml), IL-6 (32.45 (9.98–73.05) pg/ml), IL-10 (5.15 (3.68–10.45) pg/ml), TNF-α (3.15 (1.80–6.28) pg/ml) and IFN-γ(16.40 (7.48–32.95) pg/ml) concentrations did not differ significantly between the coinfection and MP groups (3.30 (2.70–3.70) pg/ml, 23.95 (6.30–190.80) pg/ml, 5.40 (3.73–10.10) pg/ml, 2.95 (1.93–14.80) pg/ml, 13.60 (7.93–32.60) pg/ml, respectively) (Fig. 1B–F).

3.3. Predictive values of the independent correlation factors in patients with coinfection

To explore the predictive values of clinical and laboratory data for MP/EBV coinfection, ROC curves were made and the cut-off values with maximum sensitivities and specificities were determined. Analysis of these ROC curves showed that fever duration, the percentage of neutrophil, CRP, IL-2, IgG, and IgA were useful for differentiating MP/EBV coinfection patients from MP infection patients (Fig. 2 and Table 5). When the cut-off values for fever duration, the percentage of neutrophil, CRP, IL-2, IgG, and IgA were set at 9.50 days, 55.75%, 11.0 mg/L, 3.35 pg/ml, 9.20 g/L, and 1.0 g/L, respectively, the sensitivity and specificity in differentiating MP/EBV coinfection from MP infection were 75.9% and 42.6%, 84.8% and 44.1%, 86.1% and 35.3%, 56.5% and 68.3%, 65.2% and 63.0%, 71.0% and 57.4%, respectively. Furthermore, multiple logistic regression analysis was performed to assess predictors which allowed the differential diagnosis of MP/EBV coinfection an MP infection. The IL-2 ≥ 3.35 pg/ml was significantly predictive regarding the differentiation between the 2 groups, with the odd ratio (OR) value of 3.677 (Table 6).

4. Discussion

Although MP and EBV are common pathogens among children,[17] coinfection of EBV and MP in respiratory tract has rarely been reported in immunocompetent patients and is not commonly recognized by clinicians. In our study, we found presenting symptoms and radiographic findings in children with MP and EBV mixed infection were nonspecific and similar to what was seen in MP infection. However, coinfection patient had prolonged fever, higher CRP, and higher incidence of pulmonary consolidation, indicating that the symptoms and physical signs

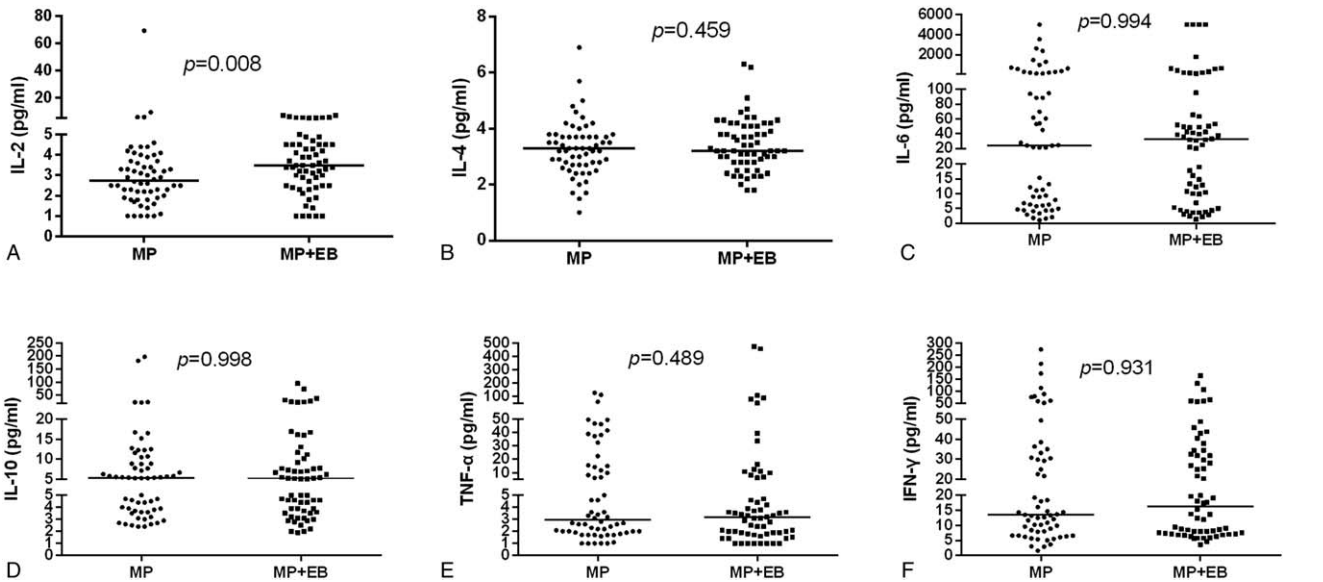

Figure 1. Comparison of serum cytokine concentrations between MP group and MP/Epstein-Barr virus coinfection group. (a) IL-2; (b) IL-4; (c) IL-6; (d) IL-10; (e) TNF-α; (f) IFN-γ. MP = *Mycoplasma pneumoniae*.

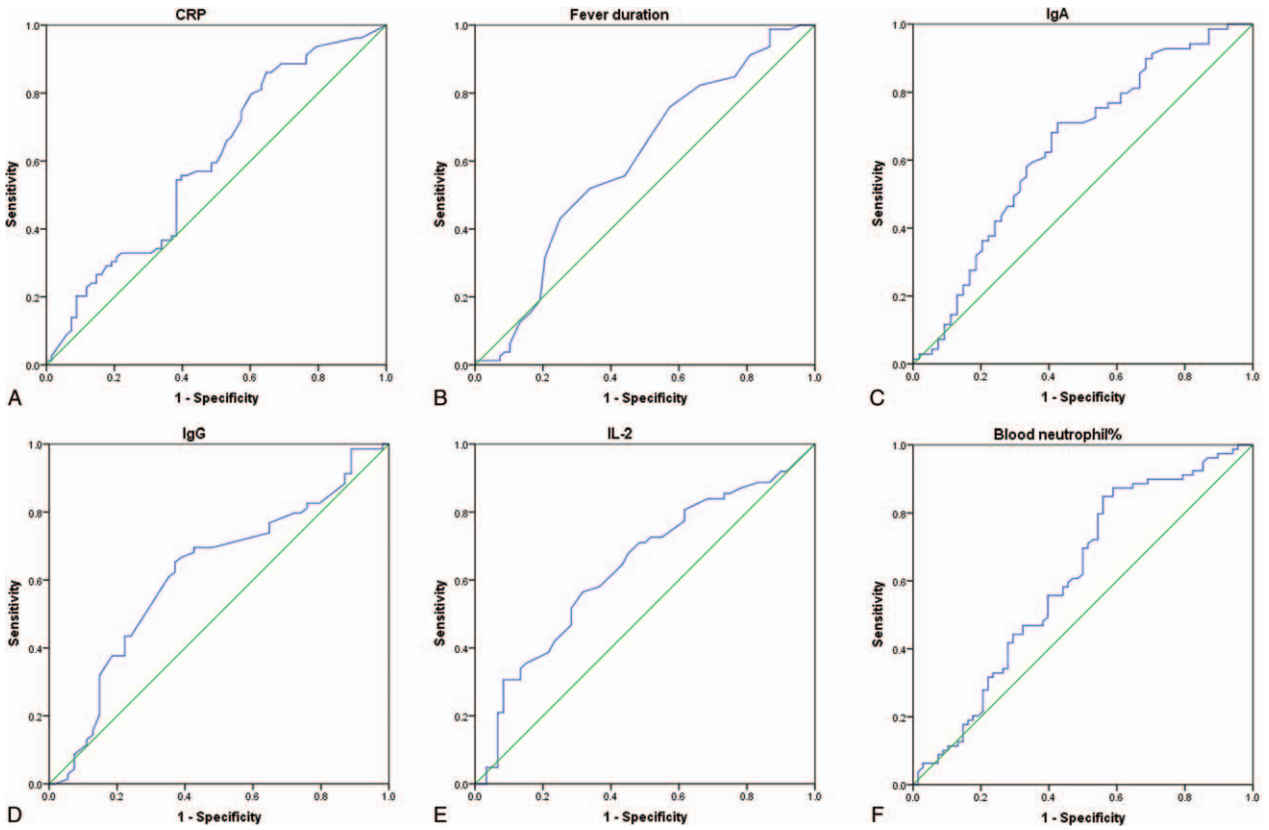

Figure 2. Receiver operator characteristic curves for differentiating *Mycoplasma pneumoniae*/Epstein-Barr virus coinfection from *Mycoplasma pneumoniae* pneumonia. (a) C-reactive protein; (b) Fever duration; (c) IgA; (d) IgG; (e) IL-2; (f) Blood neutrophil%.

seemed to be more severe in the coinfection patients. Similarly, some case studies reported patients coinfected with MP and EBV suffered more severe symptoms.[17,18] For example, Li et al presented a case of EBV and MP coinfection complicated with splenic infarction.[17] Combined leukocyte and erythrocyte agglutination was described in a 7-year-old patient with MP and EBV coinfection by Yenson et al.[18] These together indicated that to some extent MPP children with EBV coinfection had a more severe illness.

Although MP infection was usually thought to be a self-limited and benign disease, some cases may still proceed to clinical and radiological deterioration despite appropriate macrolide therapy, which were defined as RMPP. The reasons why RMPP occurred are still unclear, but it is widely accepted that excessive immune response, mixed infection, drug resistance and bacterial loads seemed play an important roles in the progress of RMPP.[19,20] Zhang et al reported that 27.0% RMPP patients had coinfection with other pathogens, such as *Streptococcus pneumoniae*, *Haemophilus influenza* and *Staphylococcus aureus*, bocavirus, rhinovirus, RSV.[9] Similarly, we showed that EBV coinfection occurred in RMPP patients, which might be a supplement to the scarcity of studies investigating coinfection of RMPP in children. Furthermore, higher incidence of RMPP was observed in MP/EBV coinfection, implying EBV coinfection in MPP patients may partially contribute to the occurrence of RMPP.

It has been reported that EBV DNA can be detected in the lung from healthy subjects, because the respiratory tract is a major reservoir for EBV.[21] Costa et al showed that EBV-DNA was positive in 40.7% transplant recipients and 23.1% nontransplant patients.[12] In our study, among the immunocompetent patients with MPP, EBV DNA was positive in nearly half of the patients. This is likely an overestimate, given that we only include MP positive cases who went through alveolar irrigation and drainage.

The pathogenic role of EBV in association with MP in lung, has not been clarified yet.[22] CRP is a gross biochemical index of inflammation and reflects the acute severe systemic inflammatory. In present study, the significant elevated serum CRP suggested the severity of systemic inflammatory responses to MP/EBV coinfection. Cell-mediated immunity and hypercytokinemia were demonstrated to play important roles in the MP infection progress.[15] Various cytokines, including IL-2, IL-8, IL-18, were reported to be involved in the immune reaction to MP infection.[23] Of interest, we found IL-2 significantly elevated in the coinfection group, showing to some extent EBV may

Table 5

ROC curve analysis for predicting MP/EBV coinfection in MPP patients.

Variables	AUC	*P* value	95% confidence interval
IL-2	0.638	.008	0.539–0.737
N% in blood	0.609	.023	0.516–0.703
CRP	0.599	.040	0.506–0.691
IgA	0.639	.008	0.539–0.740
IgG	0.618	.024	0.517–0.720
fever duration	0.599	.039	0.506–0.692

AUC = area under the curve, CRP = C-reactive protein, EBV = Epstein-Barr virus, MP = *Mycoplasma pneumoniae*, MPP = Mycoplasma pneumoniae pneumonia, ROC = receiver operating characteristics.

Table 6

Stepwise logistic regression analysis for the related factors predicting the MP/EBV coinfection in MPP patients.

Variable	B	S.E.	Wald	*P* value	OR	95%CI	
						Lower	Upper
IL-2 ≥ 3.35 (pg/ml)	1.302	0.492	7.015	.008	3.677	1.403	9.639
N% ≥ 55.75%	0.177	0.563	0.099	.753	1.194	0.396	3.598
CRP ≥ 11.0 (mg/L)	0.711	0.586	1.47	.225	2.035	0.645	6.417
fever duration ≥ 9.50 (days)	1.048	0.554	3.585	.058	2.853	0.964	8.444
IgA ≥ 1.00 (g/L)	0.987	0.522	3.576	.059	2.683	0.965	7.459
IgG ≥ 9.20 (g/L)	0.869	0.488	3.167	.075	2.384	0.916	6.204

CRP = C-reactive protein, EBV = Epstein-Barr virus, MP = *Mycoplasma pneumoniae*, MPP = Mycoplasma pneumoniae pneumonia.

accelerate the immune response of MP. Likewise, a study reported that some mycoplasma species may act as immunomodulatory cofactors by eliciting inappropriate cytokine gene expression in B cells latently infected with EBV.[24] Moreover, our study also showed that MP/EBV coinfection patients had higher incidence of pulmonary consolidation. Pulmonary consolidation is usually caused by abnormal transport of airway secretions, which is associated with excessive inflammatory reactions. These results indicated coinfection might lead to release higher cytokines, and then contribute to the excessive inflammation reaction. Additionally, IL-2, as a T cell growth factor, plays an important role in induction/suppression of immune responses via activation of regulatory T lymphocytes.[25] It has been approved for the treatment of a variety of disease, including metastatic melanoma and renal cell carcinoma, with beneficial results.[26] We showed IL-2 response to MP/EBV coinfection was higher than that to MP single infection and was defined as a risk factor of coinfection, implying IL-2 is of considerable therapeutic interest. Furthermore, ROC curve analysis showed a good discriminatory power of IL-2 for predicting MP/EBV coinfection. As a result, IL-2 might be useful for the identification of patients at high risk for MP/EBV coinfection.

This study has several limitations. Firstly, as it was a retrospective study, we collected all the qualified children rather than calculated the sample size in the study period. The sample size is not large enough. These might lead to selection bias and reduce the test efficiency. Secondly, the collection of the laboratory samples did not occur on the same day after the disease onset, which might produce bias. Thirdly, our study was based on a single center for data, which might result in potential biases and a multi-center study is need in the future. Fourthly, FOB is an invasive procedure. It was performed in relative severe MPP patients, for example, whose lung inflammation were difficult to absorb, to help diagnosis, and treatment, resulting in selection basis. However, despite these limitations, our study is the first report focus on the MP/EBV coinfection, and clearly indicated IL-2 has the potential to be used as a good predictor of MP/EBV coinfection in children with MPP. Further prospective study enrolled with a large number of patients with MPP is needed to be carried out to clarify the pathogenic role of EBV in MPP and the potential utility of IL-2 as a predictor.

5. Conclusions

Our study illustrated that MP/EBV coinfection pose a higher risk for prolonged symptoms and severe complications. Greater awareness among clinicians would ensure an early and accurate diagnosis of coinfection of MP/EBV. Further studies are needed to clarify the pathogenesis and interactions involved in MP/EBV coinfection.

Acknowledgments

We thank all patients and guardians enrolled in this study. Also, we thank all doctors and nurses who have participated in this study.

Author contributions

Data curation: Jinling Liu.
Formal analysis: Yingshuo Wang.
Investigation: Junfen Zhou, Fang Jin, Xiaoyang Chen.
Methodology: Junfen Zhou, Yingshuo Wang.
Supervision: Zhimin Chen
Validation: Zhimin Chen
Writing – original draft: Yingchun Xu, Shuxian Li
Writing – review & editing: Yingchun Xu, Shuxian Li, Jinling Liu.

References

[1] Kashyap S, Sarkar M. Mycoplasma pneumonia: clinical features and management. Lung India 2010;27:75–85.
[2] Ferwerda A, Moll HA, de Groot R. Respiratory tract infections by Mycoplasma pneumoniae in children: a review of diagnostic and therapeutic measures. Eur J Pediatr 2001;160:483–91.
[3] Juven T, Mertsola J, Waris M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J 2000;19: 293–8.
[4] Michelow IC, Olsen K, Lozano J, et al. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. Pediatrics 2004;113:701–7.
[5] Jiang W, Wu M, Zhou J, et al. Etiologic spectrum and occurrence of coinfections in children hospitalized with community-acquired pneumonia. BMC Infect Dis 2017;17:787.
[6] Oumei H, Xuefeng W, Jianping L, et al. Etiology of community-acquired pneumonia in 1500 hospitalized children. J Med Virol 2018;90:421–8.
[7] Chen CJ, Lin PY, Tsai MH, et al. Etiology of community-acquired pneumonia in hospitalized children in northern Taiwan. Pediatr Infect Dis J 2012;31:e196–201.
[8] Peng D, Zhao D, Liu J, et al. Multipathogen infections in hospitalized children with acute respiratory infections. Virol J 2009;6:155.
[9] Zhang X, Chen Z, Gu W, et al. Viral and bacterial co-infection in hospitalised children with refractory Mycoplasma pneumoniae pneumonia. Epidemiol Infect 2018;146:1384–8.
[10] Buecher C, Mardy S, Wang W, et al. Use of a multiplex PCR/RT-PCR approach to assess the viral causes of influenza-like illnesses in Cambodia during three consecutive dry seasons. J Med Virol 2010;82:1762–72.
[11] Razanajatovo NH, Richard V, Hoffmann J, et al. Viral etiology of influenza-like illnesses in Antananarivo, Madagascar, July 2008 to June 2009. PLoS One 2011;6:e17579.

[12] Costa C, Elia M, Astegiano S, et al. Quantitative detection of Epstein-Barr virus in bronchoalveolar lavage from transplant and nontransplant patients. Transplantation 2008;86:1389–94.
[13] Gautschi O, Berger C, Gubler J, et al. Acute respiratory failure and cerebral hemorrhage due to primary Epstein-Barr virus infection. Respiration 2003;70:419–22.
[14] Xu D, Li S, Chen Z, et al. Detection of *Mycoplasma pneumoniae* in different respiratory specimens. Eur J Pediatr 2011;170:851–8.
[15] Tamura A, Matsubara K, Tanaka T, et al. Methylprednisolone pulse therapy for refractory Mycoplasma pneumoniae pneumonia in children. J Infect 2008;57:223–8.
[16] Li H, Li S, Zheng J, et al. Cerebrospinal fluid Th1/Th2 cytokine profiles in children with enterovirus 71-associated meningoencephalitis. Microbiol Immunol 2015;59:152–9.
[17] Li Y, Pattan V, Syed B, et al. Splenic infarction caused by a rare coinfection of Epstein-Barr virus, cytomegalovirus, and Mycoplasma pneumoniae. Pediatr Emerg Care 2014;30:636–7.
[18] Yenson PR, Fleming A, Kaikov Y, et al. Combined neutrophil and erythrocyte agglutination in a 7-year-old boy. J Pediatr Hematol Oncol 2007;29:664–5.
[19] You SY, Jwa HJ, Yang EA, et al. Effects of methylprednisolone pulse therapy on refractory *Mycoplasma pneumoniae* pneumonia in children. Allergy Asthma Immunol Res 2014;6:22–6.
[20] Wang M, Wang Y, Yan Y, et al. Clinical and laboratory profiles of refractory Mycoplasma pneumoniae pneumonia in children. Int J Infect Dis 2014;29:18–23.
[21] Lung ML, Lam WK, So SY, et al. Evidence that respiratory tract is major reservoir for Epstein-Barr virus. Lancet 1985;1:889–92.
[22] Egan JJ, Stewart JP, Hasleton PS, et al. Epstein-Barr virus replication within pulmonary epithelial cells in cryptogenic fibrosing alveolitis. Thorax 1995;50:1234–9.
[23] Youn YS, Lee KY. Mycoplasma pneumoniae pneumonia in children. Korean J Pediatr 2012;55:42–7.
[24] Schaffner E, Opitz O, Pietsch K, et al. Human pathogenic Mycoplasma species induced cytokine gene expression in Epstein-Barr virus (EBV)-positive lymphoblastoid cell lines. Microb Pathog 1998;24:257–62.
[25] Mitra S, Leonard WJ. Biology of IL-2 and its therapeutic modulation: mechanisms and strategies. J Leukoc Biol 2018;103:643–55.
[26] Rosenberg SA. IL-2: the first effective immunotherapy for human cancer. J Immunol 2014;192:5451–8.