Profile of proinflammatory cytokines in children with *Mycoplasma pneumoniae* pneumonia

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Abstract

Background: To explore the profile and clinical significance of proinflammatory cytokines in serum and bronchoalveolar lavage fluid (BALF) of children with *Mycoplasma pneumoniae* pneumonia (MPP) and to elucidate the etiology and pathogenesis of severe MPP (SMPP).

Methods: A cohort of 108 children with MPP was divided into SMPP (n=56) and non-severe MPP (NSMPP) (n=52). A total of 40 cases of hospitalized children, who underwent elective surgery, were selected as control group. The levels of proinflammatory cytokines in the serum and BALF were measured by ELISA (Enzyme-linked immunosorbent assay).

Results: Compared to the control group, the MPP children showed that the levels of peripheral blood (PB) TNF- α , IFN- γ , GM-CSF, IL-17, IL-18, IL-36a, sB7-H1, and sB7-H3 were much higher at the acute phase ($P < 0.05$). Also, they were significantly higher in the SMPP group than that in the NSMPP group ($P < 0.05$). Furthermore, in the SMPP group, the levels of TNF- α , IFN- γ , GM-CSF, IL-1b, IL-2, IL-8, IL-36, MPO, MMP-9, NE, sB7-H3 in BALF were significantly higher at acute phase as compared to the control group ($P < 0.05$). sB7-H3 was positively correlated with major proinflammatory cytokines in both PB and BALF specimens. The level of sB7-H3 in PB was > 8000 pg/mL was an independent risk factor for SMPP.

Conclusions: Excessive inflammation plays a critical role in the occurrence and development of MPP, especially SMPP, and sB7-H3 is an independent risk factor of SMPP.

Introduction

Respiratory infections are common diseases in childhood, among which community-acquired pneumonia (CAP) is one of the leading causes of child mortality worldwide, especially in developing countries. According to the World Health Organization, in the year 2000, approximately 2–4 million children died of pneumonia worldwide. High medical expenses for the treatment of pneumonia increase the financial burden on the society and families each year [1-3]. Viruses, bacteria, and atypical pathogens are the common causes of respiratory infection in children, of which, *Mycoplasma pneumoniae* (MP) is the first pathogen detected in the hospitalized children [4]. More than 40% of CAP is caused by infection with MP, and about 18% children require hospitalization [5].

According to the previous clinical reports, *M. pneumoniae* pneumonia (MPP) is mild and self-limiting with an adequate response to the macrolide antibiotics treatment. However, recent studies demonstrated that the number of severe cases of MPP is steadily increasing [6]. Children suffering from necrotizing pneumonia accounted for about 0.5–2%

of all MPP cases [7]. Severe MPP (SMPP) includes abundant pleural effusion, acute respiratory distress syndrome (ARDS), pulmonary fibrosis, and obstructive bronchiolitis. It is life-threatening and can easily leave the lung sequelae, such as bronchiectasis, atelectasis, and bronchiolitis obliterans.

Presently, the etiology and pathogenesis of non-severe MPP (NSMPP) and SMPP are uncertain. Previous studies reported that some pathogenic factors, such as hydrogen peroxide [8] and community-acquired respiratory distress syndrome (CARDS) toxins [9], are associated with the development of pneumonia; however, these pathogenic factors cannot explain the differences in the clinical features after MP infection in children of different ages. The older children are more likely to suffer from SMPP, which might be related to the development of the immune function of the younger group that is incomplete. Therefore, we can speculate that excessive immune inflammation plays a critical role in the progression of SMPP. Recently, several studies reported that the innate and adaptive immune responses are crucial in MP infection [10-14]. The effector cells release proinflammatory cytokines to trigger a series of inflammatory cascades, which eventually leads to immune and inflammatory responses. The effector cells involved in innate immunity inflammation primarily include airway epithelial cells, alveolar macrophages, and neutrophils, while those involved in the adaptive immune inflammation are T lymphocytes, especially helper T lymphocytes (Th). Therefore, defining the expression level of the associated cytokines of effector cells in the innate and adaptive immunity in children with different severity of MPP is favorable for the assessment of the severity and prognosis of the disease and identifying the optimal time point for the administration of glucocorticoids.

Methods

Study population

This study was conducted from January 2014 to December 2015 in pediatric patients at the Department of Respiratory Disease of the Affiliated Children's Hospital, Soochow University, China. The patients were confirmed to have MP infection by the laboratory test and fulfillment of the diagnostic criteria of CAP. Finally, 108 children with MPP were enrolled. A total of 40 cases of surgical hospitalized children, underwent elective surgery ,

simultaneously, and were selected as the control group. The control group children had no history of infectious disease and use of medication in the past 4 weeks, no family history and personal anaphylactic disease, and no other allergic diseases.

This study was approved by the Audit Committee of the Children's Hospital of Soochow University in China. We confirmed that all methods were performed in accordance with the relevant guidelines and regulations. Informed consent have been obtained from the parent and/or legal guardian for study participation in the methods section.

The criteria of SMPP: Based on the diagnosis of pneumonia and 7-day macrolide antibiotic therapy invalid (after administering macrolide antibiotics for more than 1 week, children still have fever, clinical symptoms, and imaging data continued to worsen), any one of the following should be fulfilled: (1) body temperature $>38.5^{\circ}\text{C}$, severe systemic toxic symptoms; (2) significantly rapid respiration (infants breath >70 times/min, older children breaths >50 times/min); (3) dyspnea; (4) cyanosis; (5) multiple lung involvement or $\geq 2/3$ lung involvement; (6) significant pleural effusion; (7) percutaneous oxygen saturation $\leq 92\%$. Except for the children with SMPP, the remaining showed mild MPP, i.e., NSMPP. Of the 108 children with MPP, 56 were SMPP, of whom 43 were treated with bronchoscopic alveolar lavage.

MPP exclusion criteria were as follows: evidence of typical bacterial pneumonia; positive respiratory virus antigen test; presence of a risk factor for fungal pneumonia or thrush; sputum smear finding acid-fast bacilli; purified protein derivative (PPD) test indicated tuberculosis infection; presence of other systemic diseases; recurrent respiratory tract infections; congenital bronchopulmonary dysplasia; continuous usage of systemic glucocorticoids for ≥ 1 week within the past 3 months; contraindications for the use of azithromycin; pediatric patients undergoing experimental drugs or instrumentation.

Nasopharyngeal aspirate specimens and BALF collection

Nasopharyngeal aspirate specimens were collected from each study participant within 24 h after admission by applying negative pressure aseptic sputum aspiration. Fiber optic bronchoscopy and BALF collection were performed as described previously [15].

Diagnostic methods of MP infection (quantitative ELISA and fluorescent PCR)

The presence of specific IgM and IgG antibodies against *M. pneumoniae* were investigated in serum samples of patients in the acute phase of MPP (at the time of admission) and convalescent phase (on discharge), respectively, using a commercial ELISA kit (Serion ELISA classic *M. pneumoniae* IgG/IgM, Institute Virion/Serion, Würzburg, Germany) according to the manufacturer's instructions as described previously [16]. A real-time PCR procedure (Da an Gene Co. Ltd, Guangzhou, China) approved by the State Food and Drug Administration of China was used for the detection of *M. pneumoniae* as described previously [15].

Detection of multiple pathogens

Direct immunofluorescence was used to detect respiratory syncytial virus infection (RSV), influenza virus A (IVA), influenza virus B (IVB), parainfluenza virus (PIV) I, PIV II, PIV III, and adenovirus (ADV). The assay kits were purchased from Chemicon (CA, USA). Real-time PCR (Bio-Rad icycle) was used to detect the human metapneumovirus (hMPV) and human bocavirus (HBoV). RNA was extracted from the sputum specimens using TRIzol (Invitrogen, Carlsbad, CA, USA).

Determination of peripheral blood proinflammatory cytokines

The levels of IL-2, IL-4, IL-7, IL-18, IL-36 α , TNF- α , GM-CSF, TGF- β , and IFN- γ (R&D, USA), sB7-H1, sB7-H2, and sB7-H3 (Xuguang Kexing Biological Technology Co. Ltd, Suzhou, China) in serum were determined by ELISA according to the manufacturer's specifications.

Determination of BALF pro-inflammatory cytokines

The levels of IFN- γ , TNF- α , GM-CSF, IL-1 β , IL-2, IL-36, MPO, MMP-9, NE (R&D, USA) and sB7-H3 (Xuguang Kexing Biological Technology Co. Ltd, Suzhou, China) in the supernatant of BALF were measured by ELISA according to the manufacturer's instructions.

Statistical analysis

Normal distribution data were expressed as mean $\pm$ standard deviation ($\pm$ SD); the two groups were compared using the t-test. The non-normal distribution data were expressed as the median (interquartile range); the two groups were compared using the Wilcoxon rank sum test. Counting data were expressed as a rate, and the two groups were compared using

χ^2 test or Fisher's exact test. Pearson's linear correlation analyzed the correlation of normal distribution data, while Spearman linear correlation analysis was utilized for the non-normal distribution data. Statistical significance was defined as $P < 0.05$. All analyses were performed using SPSS software package (IBM, standard version 18.0).

Results

Clinical characteristics of children with MPP (NSMPP vs. SMPP)

A cohort of 108 children, aged 50.1 ± 32.8 months, was enrolled in the present study. Of these 108, 56 children exhibited SMPP (51.9%), while 52 showed NSMPP (48.1%). The age of the SMPP group was significantly older than that of the NSMPP group ($P < 0.05$). Compared to the NSMPP group, the duration of fever and the median length of the hospital stay was significantly longer in the SMPP group ($P < 0.05$).

In terms of the laboratory examinations, the median levels of the serum C-reactive protein (CRP), the median absolute count, and the percentage of neutrophils in the SMPP group were significantly higher than those in the NSMPP group ($P < 0.05$). Regarding the immune function indicators, the median levels of peripheral blood (PB) IgG and IgA in the SMPP group were significantly higher ($P < 0.05$), especially the IgGs, while no significant difference was observed in the levels of cellular immunity indicators ($P > 0.05$). In terms of bronchoalveolar lavage fluid (BALF) cytology, the proportion of neutrophils and lymphocytes in the SMPP group was significantly higher than that of the NSMPP group, while the proportion of phagocytic cells was lower ($P < 0.05$).

Moreover, all patients in the SMPP group used glucocorticoids (prednisone, methylprednisolone), while the usage rate in the NSMPP group was only 80% ($P < 0.05$). The comparison of the remaining clinical indicators is shown in Table 1.

Table 1. Comparison of clinical features between NSMPP and SMPP

Clinical features	NSMPP (n=52)	SMPP (n=56)	P-value
Population Characteristics			
Age (months)	48.4±35.4	66.6±39.0	0.038
Male (n, %)	28 (53.8)	31 (55.3)	0.9003
Clinical manifestations			
Duration of fever (days)	7.6±1.7	12.8±2.5	<0.0001
Length of hospital stay (days)	7.6±2.8	12.3±3.1	<0.0001
Laboratory examinations			
WBC (1000/mL)	8.4±4.0	8.6±3.8	0.991
N (%)	48.1±15.7	63.1±15.9	<0.0001
Absolute neutrophil count (1000/mL)	4.1±2.3	6.1±3.5	0.001
CRP (mg/L)	9.4 (0.8-12.2)	18.0 (7.7-18.4)	0.033
PLT (1000/mL)	343±121	342±92	0.96
ALT (IU/L)	14.1 (10.5-26.5)	14.1 (10.3-23.0)	0.9
LDH (IU/L)	364.7 (294.7- 448.7)	389.1 (307.5- 515.9)	0.32
CK-MB (IU/L)	23.2 (16.1-28.7)	21.4 (16.4-25.4)	0.261
IgG (g/L)	6.9±2.7	8.4±2.6	0.016
IgA (g/L)	0.8±0.7	1.2±0.7	0.03
IgM (g/L)	1.5±0.7	1.7±0.9	0.25
Subtypes of T lymphocytes (%)			
CD3+	62.9±11.4	63.4±9.8	0.852
CD3+CD4+	33±8.9	34.4±9.0	0.664
CD3-CD8+	26.2±8.0	26.1±2.9	0.934
CD3-CD19+	23.4±10.6	22.4±10.0	0.66
CD3-CD(16+56+)+	11.9±4.8	12.3±7.7	0.811
CD19+CD23+	11.6±4.9	10.4±5.4	0.327
BALF cytology (%)			
Phagocyte (%)	56.4±31.6	31.3±22.2	<0.0001

Neutrophils (%)	36.2±30	59.6±24	<0.0001
Lymphocytes (%)	7.2±6.3	9.5±5.6	0.04
Eosinophils (%)	0 (0-0)	0 (0-0)	0.115
Radiological features (n, %)			
Lobular pneumonia	29 (55.8)	18 (32.1)	0.019
Segmental or lobar pneumonia	23 (44.2)	38 (67.9)	0.019
Accompanied by pleural effusion	0 (0)	11 (19.6)	<0.0001
Major drug use			
Macrolide antibiotics (n, %)	52 (100)	56 (100)	1
Glucocorticoids (n, %)	42 (80.8)	56 (100)	<0.0001

Note: Data in the table are presented as mean ± standard deviation or quartile.

Expression of PB proinflammatory cytokines

Compared to the healthy control group, the levels of PB TNF- α , IFN- γ , GM-CSF, IL-17, IL-18, IL-36 α , sB7-H1, and sB7-H3 were much higher at the acute phase ($P<0.05$). On the other hand, the levels of IL-4 and sB7-H2 were much lower ($P<0.05$).

The levels of PB TNF- α , IFN- γ , GM-CSF, IL-17, IL-18, IL-36 α , sB7-H1, and sB7-H3 at convalescent phase were significantly lower than those at the acute phase ($P<0.05$), while that TGF- β was higher ($P<0.05$). Besides, the levels of PB TNF- α , IFN- γ , GM-CSF, IL-36 α , sB7-H1, and sB7-H3 at convalescent phase did not decline to normal, albeit were higher than those in the healthy control group ($P<0.05$) (Fig. 1).

Figure 1. Comparison of PB proinflammatory cytokine levels at acute and convalescent phases

The levels of PB TNF- α , IFN- γ , GM-CSF, IL-17, IL-18, sB7-H1, and sB7-H3 in the SMPP group were significantly higher than those in the NSMPP group ($P<0.05$) (Fig. 2).

Figure 2. Comparison of PB proinflammatory cytokine levels in the NSMPP and SMPP groups at the acute phase

Expression of proinflammatory cytokines in BALF of children with SMPP

In the SMPP group, the levels of TNF- α , IFN- γ , GM-CSF, IL-1 β , IL-2, IL-8, IL-36, MPO, MMP-9, NE, and sB7-H3 in BALF were significantly higher at the acute phase as compared to the healthy control group ($P<0.05$). After treatment, the levels of TNF- α , IFN- γ , GM-CSF, IL-1 β , IL-2, IL-8, IL-36, MPO, MMP-9, NE, and sB7-H3 were decreased considerably ($P<0.05$). However, the levels of TNF- α , IFN- γ , IL-2, MPO, NE, and sB7-H3 did not drop to normal and were higher than those in the healthy control group ($P<0.05$) (Fig. 3).

Figure 3. Comparison of expression levels of BALF proinflammatory cytokine at acute and convalescent phases

Clinical significance of expression of proinflammatory factor in PB

The duration of fever was positively correlated with the levels of PB sB7-H3, IFN- γ , and GM-CSF in children with SMPP ($P<0.05$). None of the proinflammatory factors correlated with the length of hospital stay (Fig. 4).

Figure 4. Correlations between the levels of PB IFN- γ , GM-CSF, sB7-H3, and clinical parameters in children with MPP

Clinical significance of expression of proinflammatory factor in BALF of children with SMPP

The levels of sB7-H3, MMP-9, and IL-1 in BALF were positively correlated with the length of hospital stay. In addition, IFN- γ was correlated with the duration of fever and the levels of sB7-H3, GM-CSF, MPO, NE, IL-1 β , and IL-36 were positively correlated with the lung CT values in children with SMPP ($P<0.05$) (Fig. 5).

Figure 5. Correlation between BALF pro-inflammatory cytokine expression and clinical parameters in children with SMPP

The interactions between proinflammatory factors were subjected to multivariate regression analysis to identify the factor that was closely related to the length of hospital stay. Although MMP-9 (Table 2) was the identified factor, the most relevant to the CT value was MPO (Table 3).

Table 2. Multivariate regression analysis of correlation between length of hospital stay and proinflammatory factors

Proinflammatory factors	Standard coefficient (β)	Statistical value (t)	P-value
MMP-9	0.377	2.611	0.013
sB7-H3	0.015	0.065	0.948
IL-1 β	0.217	0.952	0.347

Table 3. Multiple regression analysis of correlation between CT value and proinflammatory factors

Proinflammatory factors	Standard coefficient (β)	Statistical value (t)	P-value
MPO	0.507	3.767	0.001
sB7-H3	0.259	1.247	0.220
GM-CSF	0.311	2.015	0.051
NE	0.243	1.610	0.115
IL-1 β	0.02	0.100	0.921
IL-36	0.182	1.136	0.263

Correlations between B7-H3 and other proinflammatory cytokines

The expression of sB7-H3 was significantly increased in both PB and BALF specimens in this study. Further analysis showed that PB sB7-H3 was positively correlated with IFN- γ , GM-CSF, and IL-4 ($P < 0.05$) (Fig. 6). In BALF, sB7-H3 was positively correlated with GM-CSF, MPO, NE, IL-1 β , IL-2, and IL-36 ($P < 0.05$) (Fig. 7).

Figure 6. Correlation between sB7-H3 and IFN- γ , GM-CSF, and IL-4 in PB

Figure 7. Correlation between sB7-H3 and GM-CSF, MPO, NE, IL-1 β , IL-2, and IL-36 in BALF

Assessment of risk factors for SMPP

To assess the independent risk factors of SMPP in children, we included statistically significant clinical variables in the logistic regression analysis and expressed the results as

crude odds ratio (OR) and adjusted OR. As shown in Table 4, the duration of fever (>10 days) and PB sB7-H3 (>8000 pg/mL) were independent risk factors for the occurrence of SMPP.

Table 4. Assessment of risk factors of SMPP

Variables	SMPP (n=56), %	Crude OR (95% CI)	Adjusted OR (95% CI)	P-value
Age (>5 years)	28 (50)	1.74 (0.80–3.75)	0.625 (0.121–3.224)	0.575
Duration of fever (>10 days)	47 (83.9)	19.465(7.339–51.627)	21.483 (6.668–69.208)	<0.0001
Absolute neutrophil count (>5000/mL)	24 (42.9)	1.687 (0.765–3.725)	0.937 (0.224–3.923)	0.929
CRP (>25 mg/L)	22 (39.3)	2.412 (1.026–5.669)	1.452 (0.274–7.695)	0.661
Segmental or lobar pneumonia	34 (60.7)	1.803 (0.839–3.874)	0.967 (0.201–4.665)	0.967
sB7-H3(>8000pg/mL)	37 (66.1)	7.258 (3.055–17.246)	11.716 (1.435–95.645)	0.022
GM-CSF(>10 ⁴ pg/mL)	27 (48.2)	1.270 (0.594–2.714)	0.348 (0.055–2.199)	0.261
IFN-γ(3000 pg/mL)	29 (51.8)	1.865 (0.864–4.030)	0.758 (0.149–3.867)	0.739
IL-36α(350 pg/mL)	24 (42.9)	1.417 (0.650–3.087)	1.278 (0.320–5.105)	0.729

Discussion

The cause of SMPP may be related to the excessive inflammatory response after MP infection [17]. This study found that the average age of children with SMPP was slightly higher than that of children with NSMPP. Additionally, children with SMPP had higher

levels of IgG and IgA . These might be related to the fact that the immune function in older children tends to be perfect exhibiting severe immune inflammation after MP infection. Compared to children with NSMPP, those with SMPP suffered a prolonged duration of fever and hospitalization: 12.8 days and 12.3 days, respectively. Recently, a 10-year retrospective study by the domestic study group found that the length of hospital stay in patients with segmental or lobar MPP was 12.70 ± 4.54 days [18], which was in agreement with the results of the present study. The mechanism of fever in MPP is described as follows: MP invades the respiratory tract, causing the host to produce exogenous or endogenous pyrogen by metabolites and released toxins, such as TNF- α , IFN- γ , and IL-1 β , which act on the set temperature of the thermoregulatory center to elevate the set point, resulting in the occurrence of fever [19]. The significantly increased level of endogenous pyrogens such as TNF- α , IFN- γ , and IL-1 β in children with SMPP results in persistent fever. The neutrophil count and CRP level in PB reflects the degree of infection and inflammation. In this study, we found that the absolute counts of neutrophils and CRP levels in PB of children with SMPP were significantly higher than those in NSMPP patients, which was consistent with that obtained at the Beijing Children's Hospital [20].

Lactate dehydrogenase (LDH) might increase when the lung is infected by MP. Reportedly, the expression level of LDH in PB of children with MPP was positively correlated with the severity of MPP as shown by Kawamata et al. [21]. Simultaneously, Inamura et al. reported that LDH expression levels could be used as a reference index for the treatment of refractory MPP with glucocorticoids. If LDH ≥ 410 IU/L, glucocorticoids can be used to treat the refractory MPP [22]. However, the present study found that the levels of LDH in the SMPP and NSMPP groups did not vary significantly, which might be related to the incomplete similarity of the study subjects. The participants of this study included children with SMPP, as well as those with refractory MPP. In addition, sample capacity is also one of the reasons for the differences.

After MP infection, various cells, including macrophages, neutrophils, and lymphocytes are involved in the pathogenic process. The current study found that the proportion of neutrophils and lymphocytes in BALF increased significantly in children with SMPP, while the proportion of macrophages decreased significantly, which was consistent with the

results from the animal models [23]. On one hand, the clearance of MP in the lung primarily relied on macrophages rather than neutrophils [24]. On the other hand, excessive neutrophils produce neutrophil peroxidase, MMP-9, and neutrophil elastase, which aggravate the inflammation of the lungs [25]. Thus, the activation and aggregation of neutrophils are not conducive to the clearance of MP in the lung tissue, which aggravates the inflammatory damage.

Recently, some studies reported that in children with MPP, the levels of PB IFN- γ , IL-4, and IL-10 were significantly higher than those in healthy children; however, the level of IL-2 was significantly decreased [26]. The changes in the level of IFN- γ was consistent that the results of the present study. However, any significant change was not found in the levels of IL-4 and IL-2, which might be related to the sample capacity, age, and severity of illness in the enrolled subjects. The more severe the disease, the more pronounced Th1-type response. In contrast, those with the mild and persistent disease, the Th2-type response was more pronounced. The concentration of IFN- γ ≥ 29.05 pg/mL in PB of children with MPP is an effective predictor of refractory MPP [27]. Compared to the healthy control group, the levels of Th17 cells and IL-17 in PB of children with MPP were significantly increased, while no significant difference was detected between the level of Tregs and TGF- β 1 [28], which was in agreement with the results of this study. Ding et al. demonstrated that serum IL-10 level in children with SMPP was significantly lower than that in children with NSMPP, which suggested that the level of Th2 cells was suppressed in children with SMPP, indicating Th1-predominant immune response [29]. A recent study found that the levels of Th9 and IL-9 significantly increased in PB of children with MP and positively correlated with the severity of the disease [30]. Th differentiation of Th cells is closely related to costimulatory molecules, especially the B7 family [31-33]. Chapoval et al. reported that the expression of B7-H3 can specifically promote the secretion of Th1-type cytokine IFN- γ , thereby suggesting that B7-H3 may promote the differentiation of T-cells to Th1 [34]. In conclusion, PB proinflammatory cytokines, especially Th1, Th2, and Th17 cytokines are highly expressed in MPP children.

In order to investigate the local inflammatory response in children with SMPP, we further analyzed the expression level of proinflammatory cytokines in BALF and focused on the

expression of innate immune inflammatory factors. Compared to the healthy control group, the levels of TNF- α , IFN- γ , GM-CSF, IL-1 β , IL-2, IL-8, IL-36, MPO, MMP-9, NE, and sB7-H3 were significantly higher [35-38]. T lymphocytes in children with SMPP were significantly activated, and the expression levels of IL-6 and IL-10 in BALF were high [35]. The expression levels of IL-4 and IFN- γ in BALF were significantly higher in children with refractory MPP than those in controls [36]. In children with MPP, the level of TNF- α in BALF in the acute phase was also elevated significantly than that in control group that did not exhibit pulmonary lesions ($P < 0.05$) [37]. This study also found that BALF neutrophils and lymphocytes in children with SMPP were increased, albeit neutrophil infiltration was predominant. [36] However, only few studies have yet addressed neutrophil-related inflammatory cytokines worldwide. Thus, we speculated that MP infected the body to stimulate the airway epithelial cells and lung macrophages in order to secrete GM-CSF and IL-8, while GM-CSF and IL-8 in BALF further activated the neutrophils and induced their recruitment into the lungs. Also, simultaneously, the activated neutrophils secreted MPO, MMP-9, and NE, resulting in excessive inflammation. Therefore, the expression level of sB7-H3 in BALF was found to be positively correlated with the length of hospital stay and lung CT value in children with SMPP, which suggests that sB7-H3 may play a critical role in SMPP.

Recent studies showed that the costimulatory molecule B7-H3 played a vital role in the adaptive as well as innate immune responses [39-41]. The higher the concentration of plasma sB7-H3, the worse the sepsis patient's prognosis. In addition, B7-H3 can promote lipoprotein-stimulated monocyte-macrophage to secrete pro-inflammatory cytokines such as TNF- α and IL-6, in order to expand the inflammatory response [39]. Therefore, B7-H3 may be a key inflammatory mediator for the development of SMPP and participate in the innate and adaptive immune responses. In this study, B7-H3 was positively correlated to the major proinflammatory cytokines in both PB and BALF specimens. PB sB7-H3 > 8000 pg/mL was an independent risk factor for SMPP and was expected to become a biomarker for the occurrence and development of SMPP. These findings were in agreement with those from the previous study [42].

Conclusions

Excessive inflammation plays a critical role in the occurrence and development of MPP, especially SMPP, and sB7-H3 is an independent risk factor of SMPP. Thus, optimizing the expression profile of proinflammatory cytokines in children with MPP is conducive in finding a novel clinical immunoassay indicators in order to dynamically and comprehensively determine the condition and prognosis and provide new ideas for exploring the immune intervention with respect to MPP.

Declarations

Abbreviations

BALF:Bronchoalveolar lavage fluid ;CAP:Community-acquired pneumonia;
ELISA:Enzyme-linked immunosorbent assay;LDH:Lactate dehydrogenase;
MP:Mycoplasma pneumoniae;MPP:Mycoplasma pneumoniae pneumonia;
NSMPP:Non-severe Mycoplasma pneumoniae pneumonia;OR:Odds ratio;
PB:Peripheral blood;SMPP:Severe Mycoplasma pneumoniae pneumonia;
Th :Helper T lymphocytes.

Authors' contributions

G.D and H.S performed the data analyses and wrote the manuscript;Z.C. and Y.Y. contributed significantly to analysis and manuscript preparation;T.W,L.H and M.W contributed to the design of the study.Y.W,C.H and W.J helped perform the analysis with constructive discussions.Z.C. and Y.Y. contributed equally to this study. All authors revised the manuscript's final content. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated and analysed during this study are included in this published article.

Ethics approval and consent to participate

This study was approved by the Institutional Human Ethics Committee of the Children's Hospital of Soochow University. Written consent was obtained from the guardians on

behalf of the patients enrolled in the present study.

Competing interests

The authors declared no competing interests.

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Figures

Figure 1

Comparison of PB proinflammatory cytokine levels at acute and convalescent phases

Figure 2

Comparison of PB proinflammatory cytokine levels in the NSMPP and SMPP groups at the acute phase

Figure 3

Comparison of expression levels of BALF proinflammatory cytokine at acute and convalescent phases

Figure 4

Correlations between the levels of PB IFN- γ , GM-CSF, sB7-H3, and clinical parameters in children with MPP

Figure 5

Correlation between BALF pro-inflammatory cytokine expression and clinical parameters in children with SMPP

Figure 6

Correlation between sB7-H3 and IFN- γ , GM-CSF, and IL-4 in PB

Figure 7

Correlation between sB7-H3 and GM-CSF, MPO, NE, IL-1 β , IL-2, and IL-36 in BALF