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Serum cytokine profile of pediatric patients with laboratory confirmed pneumococcal meningitis

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ABSTRACT

Background: *Streptococcus pneumoniae* infection is a leading cause of bacterial meningitis in children with severe sequelae. Cytokines are important molecules in regulating of host inflammatory and anti-inflammatory responses. So far, the cytokine profile of bacterial meningitis caused by single pathogen has been rarely reported. The aim of this study was to explore serum cytokine profile in pediatric patients with pneumococcal meningitis (PM) and its clinical relevance which could be considered as a valuable tool for differential diagnosis of PM.

Methods: During 2015–2018, 95 children with laboratory-confirmed PM were included. Of them, 63 had serum samples at admission. Ten cytokines including TNF-α, IL-12p40, IL-17A, IL-1β, IFN-γ, GM-CSF, IL-10, CXCL-1, IL-8 and IL-13 were measured by multiplex immunoassay in sera of 63 PM patients and 55 age-matched healthy controls (HCs). Level of serum cytokines was compared with different clinical features of patients.

Results: Significantly higher level of IL-10 was observed in patients than HCs (median, 2.19 vs. 1.92 pg/mL, $p = 0.017$). Significantly lower levels of serum IL-12p40, IL-17A and IL-1β were observed in patients than HCs (median, 0.68 vs. 10.12 pg/mL, $p < 0.0001$; 1.14 vs. 1.14 pg/mL, $p = 0.004$; 1.00 vs. 5.09 pg/mL, $p < 0.0001$, respectively). No difference was found in levels of other cytokines between patients and controls. A negative correlation was noticed between percentages of blood neutrophils and concentrations of IL-10 ($p = 0.048$, $r = -0.25$). Significantly lower levels of IL-12p40 and CXCL-1 were observed in PM patients with sepsis than those without (median 0.68 vs. 1.64 pg/mL, $p = 0.026$; 7.25 vs. 12.84 pg/mL, $p = 0.043$, respectively).

Conclusions: Our results suggested that there might be significant changes in serum pro-inflammatory and anti-inflammatory cytokines in PM children and that the determination of these cytokines may have limited value for evaluation of clinical outcome of pediatric PM.

Introduction

Streptococcus pneumoniae (*S. pneumoniae*) infection is a leading cause of bacterial meningitis (BM) in children younger than 5 years, resulting in a high case-fatality rate globally [1]. In China, during follow-up after discharge, 30% of patients found to have focal neurological deficits including mental retardation and hearing impairment [2]. Colonization of *S. pneumoniae* in the mocosa of the upper respiratory tract is one prerequisite for the development of pneumococcal meningitis (PM) in the carrier. After the colonization, *S. pneumoniae* can bind to platelet-activating factor receptor (PAFR) and polymeric immunoglobulin receptor through phosphorylcholine (ChoP) and choline-binding protein A, or directly damage the epithelium through pneumolysin (Ply). The consequence is that the *S. pneumoniae* penetrates the epithelium into the blood through endocytosis, leading to the occurrence of invasive diseases. On the

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other hands, *S. pneumoniae* can also transmigrate from nasopharyngeal epithelium to the central nervous system through olfactory nerves and mastoid without going through the bloodstream [3].

Cytokines are molecules involved in the regulation of immune and inflammatory processes. When *S. pneumoniae* are killed by neutrophils or macrophages, its ply is released to form pores in the cell membrane, allowing bacterial products to access the host cell, causing the production of inflammatory cytokines and chemokines such as interleukin (IL)-1β, interferon (IFN)-γ and IL-8 [4–6]. These inflammatory cytokines may increase the expression of PAFR so that the ChoP–PAFR-mediated invasion can be amplified [7,8]. Toll-like receptors (TLRs) recognize *S. pneumoniae* through antigen-presenting cells, resulting in the release of inflammatory cytokines such as IL-17A, tumour necrosis factor (TNF)-α, IL-1β and IL-23. While recruiting neutrophils, these cytokines also participate in the process of endothelial cell damage [9–11]. Srinivasan et al. recently reported that TNF-α in cerebrospinal fluid (CSF) can be considered as a biomarker for the diagnosis of BM in infants [12]. Experiments in PM rat model have also shown that TNF-α was increased in CSF, which might be associated with BBB disruption [13]. IL-1β could stimulate the expression of a series of inflammatory factors including IL-17A, TNF-α, IL-6, IFN-γ, IL-8 and CXCL-1, and it might be associated with brain edema [14,15]. IL-23 could activate signal transducer and activator of transcription (STAT) 3, and initiate the expression of IL-17A [16]. IL-17A can participate in the promotion and synergy of the pro-inflammatory factors in the central nervous system (CNS), and stimulates the production of the downstream chemokines such as IL-8 and CXCL-1 and induces the infiltration of immune cells into the CNS, further promoting the destruction of the BBB [11]. The anti-inflammatory cytokine IL-10, however, could decrease the release of pro-inflammatory cytokines by inhibiting Th1 and/or Th17 reactions and thereby promoting tissue repair and protecting the CNS [17]. However, there were differences observed between cytokine levels induced by distinct pathogens. It was reported that elevated levels of cytokines in CSF were observed in BM patients but not in those with viral encephalitis [18]. Moreover, among subjects with BM, patients infected with *S. pneumoniae* had significantly higher levels of IFN-γ and TNF-α in CSF than those with meningococcal meningitis [19].

Recent animal experiments have also found that endothelial cells, microglia and astrocytes could produce IL-6, TNF and IL-1β before leukocyte infiltration [20]. However, the cytokine profile of patients with BM caused by *S. pneumoniae* is rarely reported, especially in children. In this present study, we aimed to determine serum levels of 10 cytokines including pro-inflammatory cytokines such as TNF-α, IL-12p40, IL-17A, IL-1β, IFN-γ and GM-CSF, anti-inflammatory cytokines such as IL-10 and IL-13 and chemokines such as CXCL-1 and IL-8 in order to understand serum cytokine profiles in pediatric patients with PM and its clinical relevance, which could be considered as a tool for differential diagnosis of PM.

Materials and methods

Human subjects

During 2015–2018, 95 children with laboratory-confirmed PM in the Department of Infectious Diseases, Beijing Children's Hospital were included in the study. All PM patients met the standards PM diagnosis established by the World Health Organization (WHO), that is, CSF or peripheral blood of *S. pneumoniae* culture and/or specific antigen are positive, coupled with any of the following symptoms: fever (axillary temperature >38.0 °C), headache, neck stiffness, disturbed consciousness, or other meningeal irritation signs. PM patients with viral meningitis were excluded [21]. Of the 95 patients with PM, 63 had serum samples and were thus included. Unfortunately, CSF samples of these patients were not available for this analysis. The median age of patients was 18 months (interquartile range (IQR), 8.5–57 months), and the male-to-female sex ratio was 2.3. All the PM patients included in the study were found to have good clinical responses (e.g, fever curve, resolution of symptoms) after 24–36 hours of empirical antibiotics therapy. Fifty-five sera randomly selected from serum samples obtained from 600 children who attended routine physical examination in 2018 were used as the control group. The median age of these 55 healthy controls (HCs) was 11 months (IQR, 5.5–30 months), and male-to-female sex ratio was 1.39. The guardians of these HCs were interviewed and did not report that these children had meningitis or other severe infectious diseases before. Written informed consent was obtained prior to the study from all guardians of the study subjects and controls in the case of young children who were unable to write or consider consent independently. All study subjects and controls were Chinese from different families and are thus considered to be unrelated.

This study was approved by the Ethics Committee of the Capital Medical University and the Beijing Children's Hospital, Beijing, China.

Sample collection

In the first three days of admission, peripheral blood samples were taken aseptically, and serum samples were obtained by centrifuging (3000 rpm, 10 min) of the blood samples and stored at −80 °C until tested.

Determination of serum cytokines

Serum levels of TNF-α, IL-12p40, IL-17A, IL-1β, Interferon-γ(IFN-γ), granulocyte-macrophage colony stimulating factor (GM-CSF), IL-10, C-X-C motif chemokine ligand 1 (CXCL-1), IL-8 and IL-13 were determined using the multiplex immunoassay based on Luminex MAGPIX system (Invitrogen, Vienna, Austria) according to the manufacturer's instructions. Each sample was performed in duplicates with 50 μL per well. The lower detection limits (LOD) for each cytokines were: TNF-α, 8.59 pg/mL; IL-12p40, 1.35 pg/mL; IL-17A, 2.27 pg/mL; IL-1β, 1.99 pg/mL; IFN-γ, 15.00 pg/mL; GM-CSF, 15.00 pg/mL; IL-10, 1.70 pg/mL; CXCL-1, 3.00 pg/mL; IL-8, 2.76 pg/mL and. IL-13, 3.37 pg/mL. Values lower than LOD were reported as being half of the LOD.

Statistical analysis

Continuous variables were presented as medians and interquartile range (IQR), and were compared by Mann-Whitney *U* test. Categorical variables were analyzed by the χ^2 test. The spearman or pearson correlation coefficient was used to evaluate the relation between different variables. A two-tailed *p* value less than 0.05 was considered as significant. All analyses were performed by SPSS 23.0 software (IBM, Armonk, NY, USA).

Results

Demographic and clinical features of patients

Clinical and laboratory information of 63 patients with laboratory-confirmed PM was summarized in Table 1. Of 63 patients, 23 were CSF culture positive for *S. pneumoniae*, 34 were both CSF positive for specific pneumococcal antigen and blood culture positive for *S. pneumoniae*, and 6 were only blood culture positive for *S. pneumoniae*.

Table 1
Clinical and laboratory information of study subjects.

Characteristics	Patients with PM[a]
Clinical features	
Fever[b]	63 (100%)
Seizures	30 (47.6%)
Sepsis	30 (47.6%)
Hearing loss	18 (28.6%)
Hospitalization time (days)	21 (16-32)
Level of consciousness at admission	
Normal consciousness	33 (52.4%)
Disturbed consciousness	30 (47.6%)
Laboratory variables	
CRP (mg/L)	78.0 (27.1–143.9)
Glucose in CSF (mmol/L)	1.1(0.3–2.6)
Proteins in CSF (mg/L)	1790.7 (812.0–2669.0)
White cell count in CSF ($\times 10^6$/L)	857.5 (136.3–2565.8)
Polymorphonuclear cells (%)	71.7 (55.0–80.0)
Lymphocytes (%)	65.0 (45.3–80.0)
White blood cell count ($\times 10^9$/L)	15.1 (9.8–20.7)
Neutrophils (%)	78.1 (53.0–89.5)
Lymphocytes (%)	17.2 (5.5–38.8)

[a] Absolute count (%) for categorical variables and median (IQR) for continuous data, unless otherwise stated.
[b] Axillary temperature >38.0 °C.

Comparison of serum cytokine profiles between PM patients and healthy controls

Significantly lower detection rates of IL-12p40, IL-17A and IL-1β were found in the PM patients compared to the HCs (number of subjects with detectable cytokine/total number of study subjects, 27/63, 42.9% vs. 54/55, 98.2%, $p < 0.001$; 0/63, 0.0% vs. 7/55, 12.7%, $p = 0.011$; 9/63, 14.3% vs. 49/55, 89.1%, $p < 0.001$, respectively). Ten of the 63 patients and 3 of the HCs had detectable TNF-α (15.9% vs. 5.5%, $p = 0.071$). None of the 63 PM patients had detectable IFN-γ and only one case had detectable IFN-γ in HCs. None of the 63 PM patients had detectable GM-CSF and IL-13 and only two cases had detectable GM-CSF and IL-13 in the HCs. Significantly lower levels of pro-inflammatory cytokines IL-12p40, IL-17A and IL-1β were observed in patients than HCs (median, 0.68 vs. 10.12 pg/mL, $p < 0.0001$; 1.14 vs. 1.14 pg/mL, $p = 0.004$; 1.00 vs. 5.09 pg/mL, $p < 0.0001$, respectively) (Table 2, Fig. 1). Significantly higher level of anti-inflammatory cytokine IL-10 was found in PM patients than HCs (median 2.19 vs. 1.92 pg/mL, $p = 0.017$) (Fig. 2). There were no significantly differences in the serum levels of IFN-γ, TNF-α, GM-CSF, IL-13, IL-8 and CXCL-1 between PM patients and HCs (Table 2, Fig. 3).

We next compared serum cytokine profiles between controls and PM patients confirmed by only CSF culture positive for *S. pneumoniae* or both CSF positive for specific pneumococcal antigen and blood culture positive for *S. pneumoniae*. For 14 patients with a clear meningitis diagnosis by CSF analysis (elevated CSF cell count, high protein and low glucose), significantly lower levels of pro-inflammatory cytokines IL-12p40, and IL-1β were still observed in patients than HCs (median, 0.68 vs. 10.12 pg/mL, $p < 0.0001$; 1.00 vs. 5.09 pg/mL, $p < 0.0001$, respectively), and significantly higher level of anti-inflammatory cytokine IL-10 was also found in PM patients than HCs (median 3.49 vs. 1.92 pg/mL, $p = 0.007$) (Supplementary Table 1). Moreover, when the data from 23 patients who were CSF culture positive for *S. pneumoniae* were analyzed, significantly lower levels of pro-inflammatory cytokines IL-12p40 and IL-1β were still observed in patients than HCs (median, 0.68 vs. 10.12 pg/mL, $p < 0.0001$; 1.00 vs. 5.09 pg/mL, $p < 0.0001$, respectively), and significantly higher level of anti-inflammatory cytokine IL-10 was still found in PM patients than HCs (median 3.03 vs. 1.92 pg/mL, $p = 0.027$) (Supplementary Table 2). For the 34 patients who were both CSF positive for specific pneumococcal antigen and blood culture positive for *S. pneumoniae*, significantly lower level of pro-inflammatory cytokine IL-12p40, IL-1β and IL-17A were observed in patients than HCs (median, 0.68 vs. 10.12 pg/mL, $p < 0.0001$; 1.00 vs. 5.09 pg/mL, $p < 0.0001$, 1.14 vs. 1.14 pg/mL, $p = 0.031$; respectively), and significantly higher levels of pro-inflammatory cytokine TNF-α and anti-inflammatory cytokine IL-10 were observed in patients than HCs (median,7.68 vs. 4.30 pg/mL, $p = 0.008$; 2.27 vs. 1.92 pg/mL, $p = 0.023$) (Supplementary Table 3). When the data from the 57 patients were combined, similar trends were also found (Supplementary Table 4).

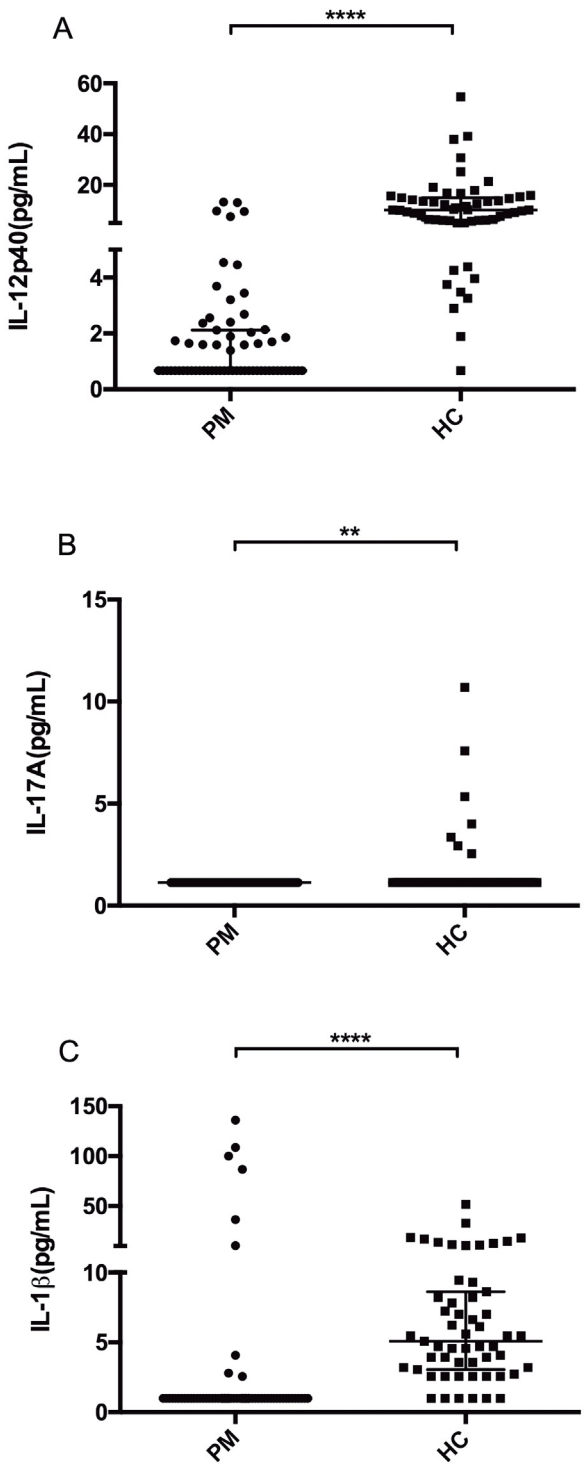

Fig. 1. Serum levels of IL-12p40, IL-17A and IL-1β in children with laboratory-confirmed PM and HCs. Data were shown as median (IQR) (**$p < 0.01$, ****$p < 0.0001$).

Table 2
Cytokine concentrations in sera of patients with laboratory confirmed PM and HCs.

Cytokines[a]	PM n = 63		HCs n = 55		*p* Value
	Median (IQR)	Range[b]	Median (IQR)	Range[b]	
IL-12p40 (pg/mL)	0.68 (0.68–2.12)	1.39–13.30	10.12 (6.06–15.00)	1.89–54.69	<0.0001
IL-17A (pg/mL)	1.14 (1.14–1.14)	–	1.14 (1.14–1.14)	2.55–10.70	0.004
IL-1β (pg/mL)	1.00 (1.00–1.00)	2.56–135.95	5.09(3.06–8.62)	2.56–51.74	<0.0001
IL-10 (pg/mL)	2.19 (0.85–4.70)	1.77–105.88	1.92 (0.85–2.39)	1.72–6.73	0.017
IFN-γ (pg/mL)	7.50 (7.50–7.50)	–	7.50 (7.50–7.50)	–	0.466
TNF-α (pg/mL)	4.30 (4.30–4.30)	15.73–87.95	4.30 (4.30–4.30)	11.24–13.84	0.052
GM-CSF (pg/mL)	7.50 (7.50–7.50)	–	7.50 (7.50–7.50)	–	0.132
IL-13 (pg/mL)	1.69 (1.69–1.69)	–	1.69 (1.69–1.69)	–	0.132
IL-8 (pg/mL)	24.19 (5.89–124.10)	2.82–2240.44	11.21 (7.10–18.36)	3.84–3772.24	0.089
CXCL-1 (pg/mL)	9.78 (4.330–19.75)	3.41–351.30	8.22 (1.50–15.49)	3.68–39.00	0.511

[a] Of ten cytokines studied, IL-17, IFN-γ, GM-CSF and IL-13 were all lower than the LOD in patients. Only one case of IFN-γ and two cases of GM-CSF and IL-13 were above the LOD in HCs.
[b] Range of positive values (values higher than the LOD).

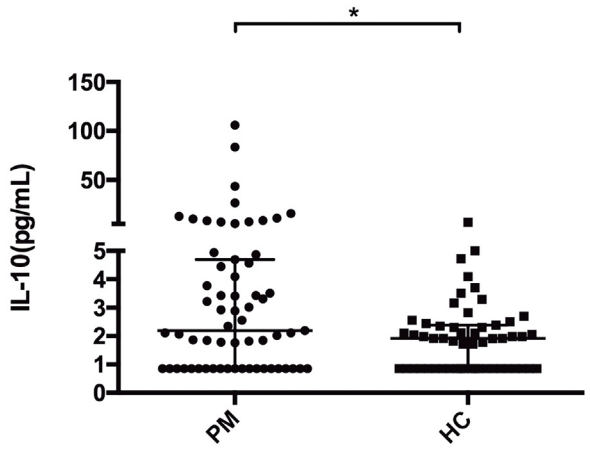

Fig. 2. Serum levels of IL-10 in patients with laboratory-confirmed PM and HCs. Data were shown as median (IQR) (*$p < 0.05$).

Correlation between level of cytokines and clinical features of patients

We evaluated the possible associations of levels of serum cytokines with glucose in CSF, proteins in CSF, white cell count in CSF, polymorphonuclear cells in CSF, lymphocytes in CSF and in blood, white blood cell count in blood, neutrophils in blood and C-reactive protein (CRP) listed in Table 1. A negative correlation was noticed between percentages of blood neutrophils and concentrations of IL-10 ($p = 0.048$, $r = -0.25$). Besides, significantly lower levels of IL-12p40 and CXCL-1 were observed in PM patients with sepsis than those without (median 0.68 vs. 1.64 pg/mL, $p = 0.026$; 7.25 vs. 12.84 pg/mL, $p = 0.043$, respectively) (Fig. 4). No significant differences were observed in other cytokines or chemokines between PM patients with and without seizures, sepsis and hearing loss.

Discussion

In this study, we compared serum cytokine profiles between pediatric patients with laboratory-confirmed PM and age-matched HCs and evaluated the association of these serum cytokine levels with clinical outcome of patients. All patients had undetectable serum IL-13 and IFN-γ. It is known that IL-13 promotes the proliferation and differentiation of B cells, and IFN-γ induced by IL-12 can increase the production of IgG2 [22]. Immunoglobulin secretion by B cells is a direct result of cytokine-induced responses. Insufficient expression of IL-13, IFN-γ and IL-12p40 in these children may influence activation and differentiation of B cells and result in the insufficient production of immunoglobulin. In fact, one third (21/63) patients studied had been treated with gamma globulin because of persistent high fever, CRP and/or white blood cell count. Recognition of bacteria by the innate immune system leads to the release of pro-inflammatory cytokines. Interleukin-23, which shares the same p40 subunit with IL-12, promotes the stabilization of IL-17A in early stages of infection. Production of IL-17A further activates nuclear factor-kappa B (NF-κB) signaling pathway, and thus induces the production of downstream inflammatory cytokines such as TNF-α, IL-1β, GM-CSF, IL-8 and CXCL-1, which promote neutrophil recruitment and activation [23]. In this study, we found that the serum levels of IL-12p40, IL-17A and IL-1β were significantly lower in children with PM compared to controls, suggesting a down-regulated activation of the NF-κB signaling pathway. Since crucial steps of activating the pathway depend on antigen presentation and T cell differentiation, which require a complement-sufficient environment, insufficient expression of IL-12p40, IL-17A and IL-1β might imply a lack of complement to some extent [24]. Notably, a recent study reported that about one third of children with invasive pneumococcal disease have meningitis, and these children most likely suffer primary immunodeficiency (PID) like deficiency of immunoglobulin, pneumococcal antibody and complements (C2 and C3) [25]. It remains to be shown how many patients in this study have PID.

Srinivasan et al. recently reported that level of CSF IL-23 was positively correlated with CSF white blood cell and protein values, and negatively correlated with CSF glucose levels in infants with BM [26]. However, serum IL-23 was not determined in this study. We measured serum IL-12p40 in these subjects and found that only 27 (42.9%) subjects had detectable IL-12p40. Interestingly, serum level of IL-12p40 was significantly lower in PM children with sepsis than those without. It should be kept in mind that the sample type used between the two studies was different.

Ye et al. compared the levels of six cytokines including IFN-γ, IL-2, IL-4, IL-6, IL-10 and TNF-α in both CSF and serum samples between Chinese pediatric patients with BM and controls [27]. Of 140 patients with BM, 20 were caused by *S. pneumoniae*. Significant difference was only found in IL-6 and IL-10 in CSF but not in serum samples between patients and controls. Xu et al. also found elevated IL-6, TNF-α, IFN-γ and IL-10 in CSF but not in serum samples between BM patients and controls, indicating meningeal inflammation could trigger a unified pro- and anti-inflammatory response [28]. Similar to their findings, we found an increased level of IL-10 in patients in this study. Further, serum concentrations of IL-10 observed in these PM patients were inversely correlated with percentages of blood neutrophils, suggesting that IL-10 could play an important role in the modulation of neutrophils

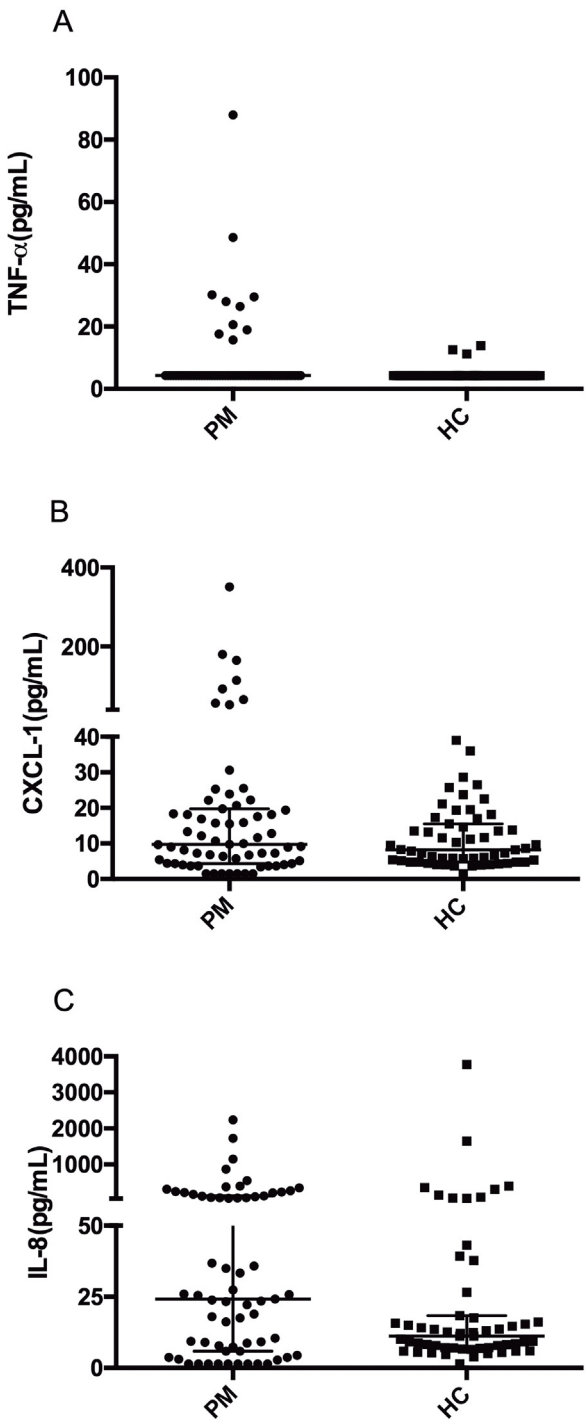

Fig. 3. Serum levels of TNF-α, CXCL-1 and IL-8 in PM children and HCs. Data were shown as median (IQR).

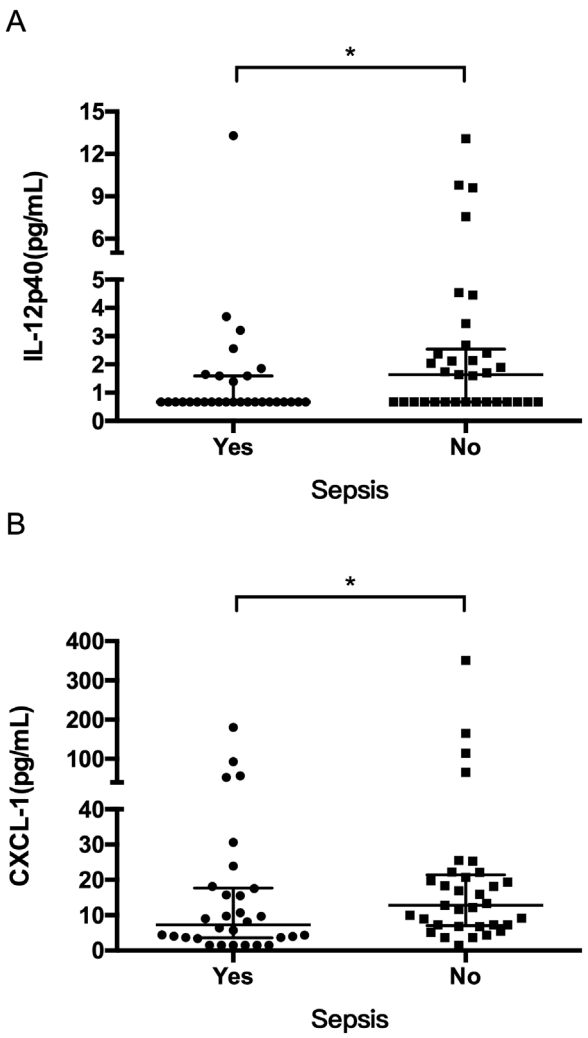

Fig. 4. Serum levels of IL-12p40 and CXCL-1 in PM children with and without sepsis. Data were shown as median (IQR) (*$p < 0.05$).

recruitment and anti-inflammation during PM. A recent animal study showed that IL-10 deficiency can increase expression of pro-inflammatory cytokines such as IL-6, TNF-α and IL-1β, cause exacerbated neutrophil infiltration, reduce load of *S. pneumoniae*, but can result in increased mortality due to immuno-pathological damage [29].

Our present study has several limitations. First, the sample size was small and only two third of studied patients had serum samples. Second, CSF samples were not available for cytokine measurement and thus a direct comparison in cytokines between serum and CSF samples was not possible. Third, although blood samples were collected within the first few days after admission, 28 children had already been treated with antibiotics before hospitalization and 42 children had been treated with glucocorticoid during hospitalization. However, no significant differences were found in these cytokines between the patients who were treated with or without antibiotics or glucocorticoid. Fourth, since the onset of disease at the time of blood sampling was unknown among the study subjects, we cannot exclude the possibility that some blood samples might not be taken in acute phase and concentrations of some inflammatory cytokines were thus not in peak. However, all the PM patients had fever (axillary temperature >38.0 °C) and obvious positive signs such as headache, abnormal consciousness and nuchal rigidity when sampling. Fifth, the pneumococcal colonisation status of the healthy controls in this study were unknown and its possible effect on production of serum cytokines and chemokines determined in this study was not clear.

Conclusions

Our results suggested that there might be significant changes in serum pro-inflammatory and anti-inflammatory cytokines in PM children and that the determination of these cytokines may have limited value for evaluation of clinical outcome of pediatric PM.

Authors' contributions

All authors contributed to the study conception and design. Material preparation and data analysis were performed by Kai Zheng, Ning Chen, Liang Zhu, Yiwei Ding and Xixi Zhang. Study subjects recruitment and clinical data collection were performed by Liang Zhu, Yiwei Ding and Xixi Zhang. Clinical part of this study was supervised by Gang Liu. The first draft of the manuscript was written by Kai Zheng. Project supervision and manuscript finalization were performed by Qiushui He. All authors read and approved the final manuscript.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

Not applicable

Availability of data and material

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

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Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval wasgranted by the Ethics Committee of the Capital Medical University and the Beijing Children's Hospital, Beijing, China [No. 2018-k-100].

Disclosure of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

We would like to thank all study subjects and their parents/guardians/relatives who agreed to participate in this study. The language of this manuscript has been checked by a native speaker of English.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jiph.2021.01.010.

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