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Plasma soluble B7-H3 levels for severity evaluation in pediatric patients with *Mycoplasma pneumoniae* pneumonia

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ABSTRACT

Seeking for the novel biomarkers for *Mycoplasma pneumoniae* pneumonia (MPP) could be not only helpful for disease diagnosis but also useful for treatment efficacy monitoring. The aim of present study was to evaluate the role of plasma soluble B7-H3 (sB7-H3) in MPP diagnosis and treatment efficacy prediction, and involvement of B7-H3 in MPP disease course. A total of 108 MPP patients and 40 control subjects were recruited into this study for changes of sB7-H3 levels in MPP. In addition, a mouse model of MPP was also established for confirmation of the involvement of sB7-H3 in MPP in vivo. Significantly increased levels of sB7-H3 were found in both mild and severe MPP patients compared to control patients. Moreover, significantly increased level of sB7-H3 was also found in severe MPP patients compared to mild subjects. The ROC curve showed sB7-H3 had severity prediction capacity in mild and severe MPP. Plasma sB7-H3 correlated positively with IFN-r and GM-CSF in mild or severe MPP patients. Moreover, significantly increased level of plasma sB7-H3 level were found in acute phase MPP patients compared to control subjects, whereas significantly decreased level of plasma sB7-H3 was found in recovery phase MPP patients compared to acute phase patients. In addition, decreased levels of sB7-H3 were found in mice from Dexamethasone group compared to LAMP group. Plasma sB7-H3 level might serve as biomarker for severity MPP prediction and treatment efficacy evaluation. Furthermore, direct involvement of B7-H3 was confirmed in vivo during the MPP disease course.

1. Introduction

Mycoplasma pneumoniae (*M. pneumoniae*), which causes mycoplasmal pneumonia in human, is one of the most important etiologic agents that causes community-acquired pneumonia (CAP) in children, and accounts for 10–40% of CAP cases [1–3]. Recent studies have shown that an increasing number of *Mycoplasma pneumoniae* pneumonia were found in Chinese children [4,5], and therefore it is necessary to novel biomarker that facilitate diagnosis and treatment monitoring in MPP.

Generally, the immune system in children is relative immature compared with that in young adults. It is reported that the symptoms of pneumonia caused by *M. pneumoniae* are correlated with the induction of pro-inflammatory cytokines [6]. These findings suggest that the excessive immune responses induced by *M. pneumoniae* play an important role in the development of pneumonia [7–9]. Costimulatory molecule B7-H3, as novel member of B7 family, was shown to play an important role in regulating the immune system, including both adaptive and innate immune responses [10]. Furthermore, involvement of B7-H3 in severe lung disease, such as acute respiratory distress syndrome (ARDS) [11], has been proved. However, the role of B7-H3 in MPP has not been fully explored.

In present study, peripheral blood samples were collected from pediatric MPP patients to evaluate the change of B7-H3 compared to control subjects. Further roles of disease severity and treatment efficacy prediction of sB7-H3 was also evaluated based on mild and severe MPP grouping, and pre- and post-treatment comparisons. In addition, a mouse model of MPP was also established for confirmation of the involvement of sB7-H3 in MPP in vivo. Our results indicated that plasma sB7-H3 level might serve as biomarker for severity MPP prediction and treatment efficacy evaluation. Furthermore, direct involvement of B7-H3 was confirmed in vivo during the MPP disease course.

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Table 1
The basic information of the plasma samples from Children's Hospital of Soochow University.

	Control	Mild MPP	Severe MPP
	(n = 40)	(n = 52)	(n = 56)
Gender (female/male)	20/20	26/26	30/26
Median age (range), year	4.34 (1.61–7.07)	3.77 (1.00–6.54)	4.87 (2.26–7.48)
Diagnosis of lobar pneumonia (n,%)	–	9 (17.3%)	16 (28.6%)
Diagnosis of lobar bronchopneumonia (n,%)	–	43 (82.7%)	40 (71.4%)
Timing of plasma collection (n,%)			
Before treatment	40 (100%)	52 (100%)	56 (100%)
After treatment	0	38 (73.1%)	40 (71.4%)

2. Methods

2.1. Patients and study design

A total of 108 children who were admitted into the Department of Respiratory of Children's Hospital of Soochow University, Suzhou, China, according to the diagnostic criteria of MPP [12], between June 2015 and December 2015 were recruited into this study (Table 1). The diagnosis of MP infection was based on serologic testing and confirmed by polymerase chain reaction (PCR) tests of nasopharyngeal secretions. The peripheral blood samples collected from all the 108 children within 24 h after hospitalization were designated as the acute phase group, while the peripheral blood samples from 78 children in 24 h before discharge were designated as the recovery group. In addition, forty children who underwent elective surgery in our hospital, with no history of infectious diseases and did not use any drugs in nearly four weeks, no family or personal history of allergic disease and other allergic diseases, were enrolled in this study as Control group.

The mild and the severe community-acquired pneumonia were defined based on the previously described criteria [13,14]. Mild MPP was defined as follows: respiratory rate < 70 breaths/min at age < 3 years old or respiratory rate < 50 breaths/min at age ≥ 3 years old, normal food-intake, no dehydration, whereas severe MPP possess following features: respiratory rate ≥ 70 breaths/min at age < 3 years old or respiratory rate > 50 breaths/min at age ≥ 3 years old, cyanosis, flaring of the nares, marked retractions, anorexia, dehydration, pleural effusion, lung necrosis/lung abscess and other complications in lung. Fifty-two and fifty-six pediatric patients were respectively grouped into mild MPP group and severe MPP group. Mild MPP patients were treated with Azithromycin (10 mg/kg·d), while severe MPP was treated with both Azithromycin (10 mg/kg·d) and an intermediate-acting glucocorticoid Methylprednisolone (1–2 mg/kg·d).

This study was approved by the Institutional Research Ethics Committee of Children's Hospital of Soochow University for clinical investigation, and the written informed consent was obtained from parents or guardians of the recruited children prior to enrollment.

2.2. Plasma cytokines measurement in MPP patients and mice

For patients, plasma samples of 108 MPP subjects and 40 control subjected were collected. Mice BALF samples were obtained from lipid-associated membrane proteins (LAMPs)-induced MPP mice as described below. The plasma level of sB7-H3 was determined by using enzyme linked immunosorbent assay (ELISA) kits (Suzhou Xuguang Kexing Biological Technology Co. Ltd., Suzhou, China) [15–17]. The human inflammatory cytokines INF-γ, IL-4, GM-CSF, and IL-17 in the plasma were detected by ELISA kits (R&D Systems). The mouse inflammatory cytokines INF-γ, IL-4, GM-CSF, and IL-17 in the BALF were measured using ELISA kits (R&D Systems).

2.3. Extraction of LAMPs from Mycoplasma

The MP standard strains M129 (Institute of pathogenic biology, University of South China) were cultured with Pleuropneumonia-Like Organisms (PPLO) broth medium (BD Biosciences) under 37 °C for 5 or 7 days. Once the color of medium changed to orange or yellow, adequate amount of bacteria liquid was transferred to new PPLO broth medium for amplification, the MP bacteria was ready for collection when the color of medium turned into orange or even yellow and maintained clear liquid. LAMPs were extracted from these MP bacteria as described previously [18]. After that, LAMPs were resuspended in PBS for protein level quantification by using an enhanced BCA protein assay kit (Beyotime Biotech, Nantong, China). Finally, all the resuspended LAMPs were aliquot into 500 μL and stored in −80 °C freezer before further experiment.

2.4. LAMPs-induced MPP model

Eight-week-old male BALB/c mice were purchased from the Chinese Academy of Sciences. All animal studies were approved by the institutional animal care and use committee at Children's Hospital of Soochow University and the experiment procedures were carried out in accordance with the international guidelines of animal welfare act. Briefly, mice were randomly divided into three groups: Control group (PBS), MPP group (LAMPs), and Dexamethasone group (LAMPs + Dexamethasone) ($n = 6$ per group). Mice in MPP group and Control group were respectively received 50 μL MPP (50 μg MPP in 50 μL PBS) and 50 μL PBS in a similar manner as described previously [19]. Mice in Dexamethasone group received Dexamethasone (1 mg/kg) intraperitoneal injection after LAMPs inhalation. The experiment was terminated at 12 h, 24 h, and 48 h after LAMPs inhalation, and lung tissues and BALF were harvested and collected thereafter. The whole procedure was performed 3 time in an independent manner.

2.5. BALF collection, protein level quantification and leukocyte counting

For BALF samples collection, the lungs of mice from above groups were lavage with 500 μL PBS for three times at 12 h, 24 h, and 48 h as described previously [20]. The total protein in the BALF were measured using an enhanced BCA protein assay kit (Beyotime Biotech, Nantong, China). Total leukocyte number in BALF were counted using TC20 cell counter (Bio-Rad, CA, USA).

2.6. Histology

Lung tissues of above mice were excised at 12 h, 24 h, and 48 h after treatment. And then the lungs were fixed in 10% formalin at room temperature. After fixation, lungs were embedded in paraffin, cut into 3 μm sections, stained with hematoxylin and eosin (H&E) and followed by microscopy examination.

2.7. Statistical analysis

Statistical analyses were performed using Graphpad Prism 5.0 or SPSS software (version 22.0). Numeration data was compared using the chi-square test, and the Student *t*-test was employed for the comparisons between groups after normal distribution confirmation of the data using Mann-Whitney *U* test. The nonparametric Spearman rank correlation test was used to analysis correlations between plasma sB7-H3 and inflammatory cytokines. A p-value of < 0.05 was considered to be statistically significant.

3. Results

3.1. Increased levels of sB7-H3 were found in plasma from MPP patients

In order to explore the clinical significance of sB7-H3 in MPP patients, we firstly evaluated the sB7-H3 level by using ELISA (Fig. S1). As shown in Fig. 1, significantly increased levels of sB7-H3 were found in both mild ($p < 0.01$) and severe ($p < 0.001$) MPP patients compared to control patients. Moreover, significantly increased level of sB7-H3 were also found in severe MPP patients compared to mild subjects ($p < 0.001$). In addition, we also examined cytokines IFN-γ, IL-4, IL-17 and GM-CSF which was produced and secreted by Th cells or endothelial cells. Similar increased manner of IFN-γ, IL-17 and GM-CSF were found in mild MPP patients compared to severe MPP subjects. Furthermore, as compared with the control subjects, in both mild and severe MPP patients, level of INF-γ, IL-17, and GM-CSF were higher and IL-4 were lower. These results demonstrated similar pattern of cytokines INF-γ, IL-17 and GM-CSF with sB7-H3, which indicated possible role of sB7-H3 in MPP diagnosis.

3.2. Severity prediction of sB7-H3 in MPP

Due to the observation of increased pattern of sB7-H3 and inflammatory cytokines in MPP patients, we further determined the severity prediction of these molecules by ROC curve (Fig. 2). The results showed that sB7-H3 (AUC = 0.7248, $p < 0.0001$) together with inflammatory cytokine IFN-γ (AUC = 0.6567, $p = 0.0063$), IL-17 (AUC = 0.6877, $p = 0.0008$) and GM-CSF (AUC = 0.6546, $p = 0.0059$), but not IL-4 (AUC = 0.5067, $p = 0.9046$), exhibited severity prediction capacity in differentiation of mild and severe MPP. Based on these ROC analysis results, sB7-H3 could be employed as the biomarker for MPP severity prediction.

3.3. Correlation between plasma sB7-H3 and inflammatory cytokines in MPP

Since the observation of the similar pattern of sB7-H3 and inflammatory cytokines, we further calculated the correlation between sB7-H3 and above examined inflammatory cytokines. As shown in Fig. 3, positive correlations were found between plasma sB7-H3 levels in mild or severe MPP patients and IFN-γ (mild: $r = 0.4715$, $p = 0.004$; severe: $r = 0.8492$, $p < 0.001$) and GM-CSF (mild: $r = 0.6782$, $p < 0.001$; severe: $r = 0.7209$, $p < 0.001$) in those patients, respectively. However, no correlation was found between plasma sB7-H3 levels in mild or severe MPP and IL-17 and IL-4. These data demonstrated that possible relationship between sB7-H3 with IFN-γ and GM-CSF.

3.4. Changes of plasma sB7-H3 level in MPP patients during the treatment course

After evaluation of the clinical significance of plasma sB7-H3 level at pre-treatment time point, we further compared plasma sB7-H3 levels during the different disease course. As shown in Fig. 4, significantly increased level of plasma sB7-H3 level were found in acute phase MPP patients compared to control subjects, whereas significantly decreased level of plasma sB7-H3 was found in recovery phase MPP patients compared to acute phase patients. Similar calculations were also performed by using the inflammatory cytokines, and GM-CSF was confirmed as the cytokine exhibiting a similar pattern as plasma sB7-H3. We further assessed the sB7-H3 level for the same patient at different stages. Clinical treatment resulted in significantly down-regulation of sB7-H3 ($p < 0.05$ versus acute phase) (Fig. S2A), which correlated with decrease of IFN-γ and GM-CSF expression ($p < 0.05$, $p < 0.01$ versus acute phase) (Fig. S2). Notably, ROC curves were constructed to determine prognosis prediction ability using plasma sB7-H3 and GM-CSF, and the AUC of sB7-H3 and GM-CSF was 0.6280 (Fig. 4F, $p = 0.0029$) and 0.6682 (Fig. 4F, $p = 0.0001$), respectively. Therefore, these results indicated that sB7-H3 and GM-CSF could be considered as a parameter of prognosis prediction for MPP.

3.5. Downregulated sB7-H3 was observed in LAMPs-induced MPP treated by dexamethasone

The plasma cytokine level might only provide indirect evidence of

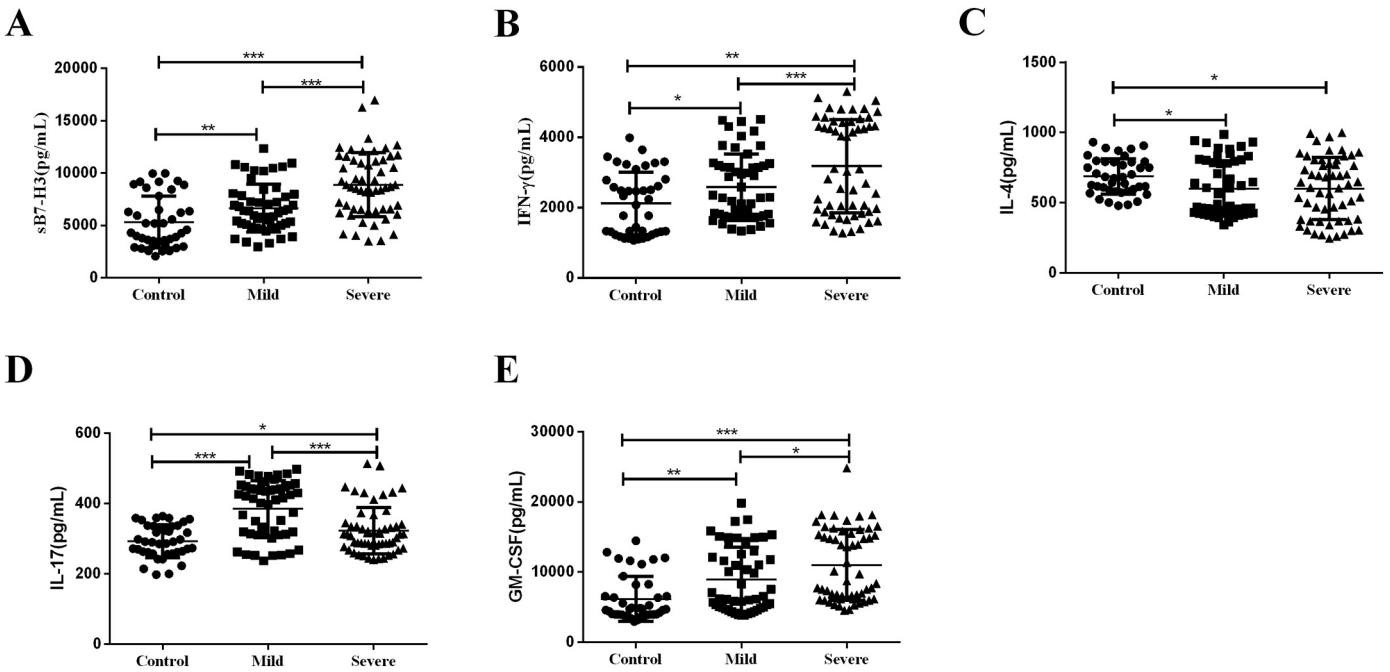

Fig. 1. Increased levels of soluble B7-H3 were found in plasma from pediatric patients with Mycoplasma pneumoniae pneumonia (MPP). The peripheral blood samples were collected from controls ($n = 40$) and pediatric patients mild MPP group ($n = 52$) and severe MPP group ($n = 56$). The plasma level of sB7-H3 (A), IFN-γ (B), IL-4 (C), IL-17 (D), and GM-CSF (E) were measured by ELISA and compared as described in the Methods. *P* value: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

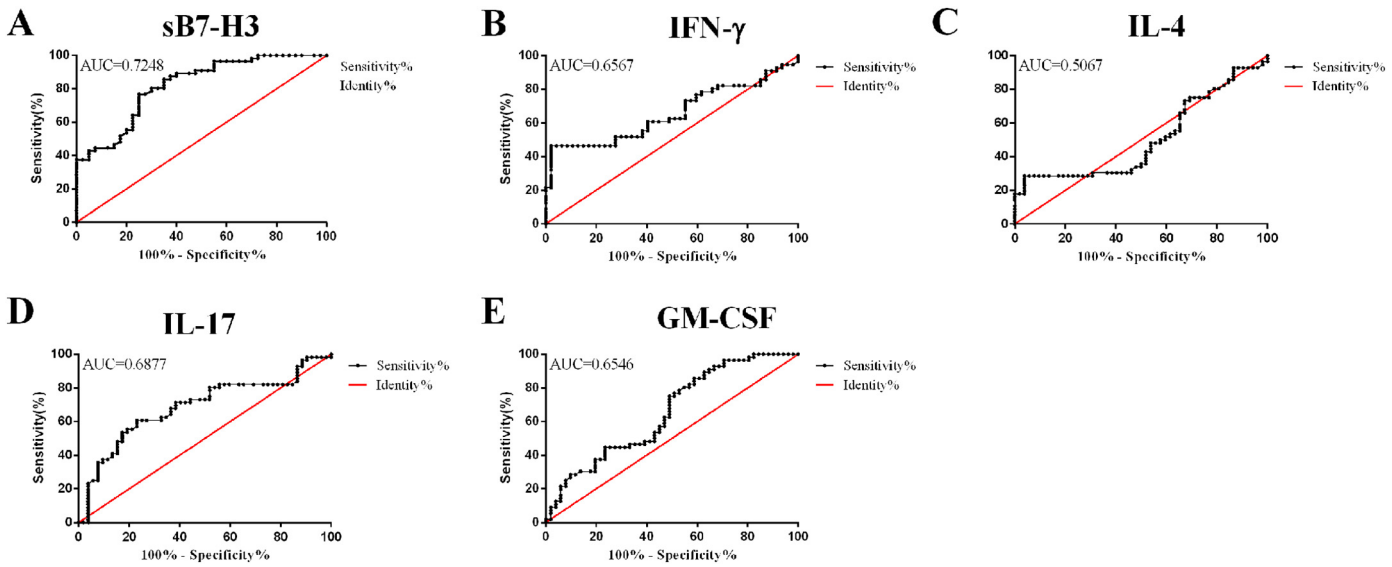

Fig. 2. Severity prediction value of plasma soluble B7-H3 and related inflammatory cytokine in pediatric patients with Mycoplasma pneumoniae pneumonia (MPP). Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic value of sB7-H3 (A) (AUC = 0.7248, $p < 0.0001$), IFN-γ (B) (AUC = 0.6567, $p = 0.0063$), IL-4 (C) (AUC = 0.5067, $p = 0.9046$), IL-17 (D) (AUC = 0.6877, $p = 0.0008$), and GM-CSF (E) (AUC = 0.6546, $p = 0.0059$) in differentiating mild and severe MPP. Soluble B7-H3 showed the best severity prediction value for MPP.

the involvement of B7-H3 in MPP, and difficult to obtain BALF from MPP patients initiated the necessity to establish a mouse model of MPP to examine the B7-H3 level in vivo.

MPP was induced by intranasal instillation of LAMPs as described in the Methods. Lung histological changes were assessed 24 h after LAMPs inhalation. Representative images of hematoxylin and eosin stained sections of lung tissues for PBS (Fig. 5A, up), LAMPs (Fig. 5A, middle), and LAMPs + Dexamethasone (Fig. 5A, down) are shown, indicating that treatment with Dexamethasone strongly ameliorates LAMPs-induced lung edema, haemorrhage, alveolar collapse, and PMN infiltration (Fig. 5A). Moreover, significantly enhanced influx of leukocytes ($p < 0.01$) into the lungs was observed in mice at 48 h after receiving an intranasal instillation of LAMPs compared with mice received an intranasal instillation of PBS (Fig. 5B). Administration of Dexamethasone after LAMPs inhalation substantially attenuated LAMPs-induced leukocyte infiltration in the lungs (Fig. 5B, $p < 0.01$ versus LAMPs-treated mice). Notably, histological examination of the lung tissues showed decreased levels of B7-H3 were found in mice from Dexamethasone group compared to LAMPs-induced group. These data provide direct involvement evidences of B7-H3 during MPP.

Following LAMPs inhalation, there were significant increases in proinflammatory cytokines IFN-γ, IL-17 and GM-CSF at mRNA levels in BALF ($p < 0.01$ versus PBS-treated mice) (Fig. 5D–F). Furthermore, Dexamethasone significantly attenuated LAMPs-stimulated IFN-γ and GM-CSF mRNA expression ($p < 0.05$, $p < 0.01$ versus LAMPs-treated mice) (Fig. 5D, F), but it had no inhibitory effects on LAMPs-stimulated IL-17 expression (Fig. 5E).

4. Discussion

MPP is a mild and self-limiting disease, however its severe MPP may result in life-threatening events and serious complications, such as pulmonary sequelae, encephalitis, myocarditis, hepatitis and nephritis. Seeking for the novel biomarkers for *Mycoplasma pneumoniae* pneumonia (MPP) could be not only helpful for disease diagnosis but also useful for treatment efficacy monitoring. In present study, we included 108 MPP patients and 40 control subjects for the role of sB7-H3 levels evaluation in MPP. Our results showed that significantly increased levels of sB7-H3 were found in both mild ($p < 0.01$) and severe ($p < 0.001$) MPP patients compared to control patients. Moreover, significantly increased level of sB7-H3 were also found in severe MPP patients compared to mild subjects ($p < 0.001$). The ROC curve showed sB7-H3 (AUC = 0.7248, $p < 0.0001$) had severity prediction capacity in mild and severe MPP. Furthermore, positive correlations were found between plasma sB7-H3 levels in mild or severe MPP patients IFN-γ (mild: $r = 0.4715$, $p = 0.004$; severe: $r = 0.8492$, $p < 0.001$) and GM-CSF (mild: $r = 0.6782$, $p < 0.001$; severe: $r = 0.7209$, $p < 0.001$), respectively. Moreover, significantly increased level of plasma sB7-H3 level were found in acute phase MPP patients compared to control subjects, whereas significantly decreased level of plasma sB7-H3 was found in recovery phase MPP patients compared to acute phase patients. In addition, a mouse model of MPP was also established for confirmation of the involvement of sB7-H3 in MPP in vivo and the results suggested that decreased levels of sB7-H3 were found in mice from Dexamethasone group compared to LAMP group.

B7-H3, an important member of the B7 superfamily of costimulatory proteins, plays an important role in both adaptive immune and innate immune. B7-H3 regulates the adaptive immune response by functioning as both a T cell co-stimulator and co-inhibitor on the one hand [21,22], on the other hand participates in the innate immune response by acting as a costimulatory factor to augment inflammatory responses during sepsis and pneumococcal meningitis [11,23]. Moreover, our previously studies have showed that B7-H3 possesses a role during MPP [16,17]. Here, we found that significantly increased levels of sB7-H3 were found in both mild ($p < 0.01$) and severe ($p < 0.001$) MPP patients compared to control patients. Moreover, significantly increased level of sB7-H3 was also found in severe MPP patients compared to mild subjects ($p < 0.001$). The ROC curve showed sB7-H3 (AUC = 0.7248, $p < 0.0001$) had severity prediction capacity in mild and severe MPP. Thus, B7-H3 was considered to be involved in pathogenesis of MPP and might be a useful biomarker of severe MPP.

The excessive immune responses have been shown to play an important role in the development of MPP [24]. As the critical player during the immune responses, cytokines are considered as the promising candidates of MPP biomarker. Here, we also examined the changes of different cytokines during in the different severity form of MPP and at pre- and post-treatment. IFN-γ, a pro-inflammatory cytokine secreted by activated T cells and NK cells [25], could also be produced by neutrophils early during acute *S. pneumoniae* pneumonia and has the capacity to induce transcription of target genes in the lungs,

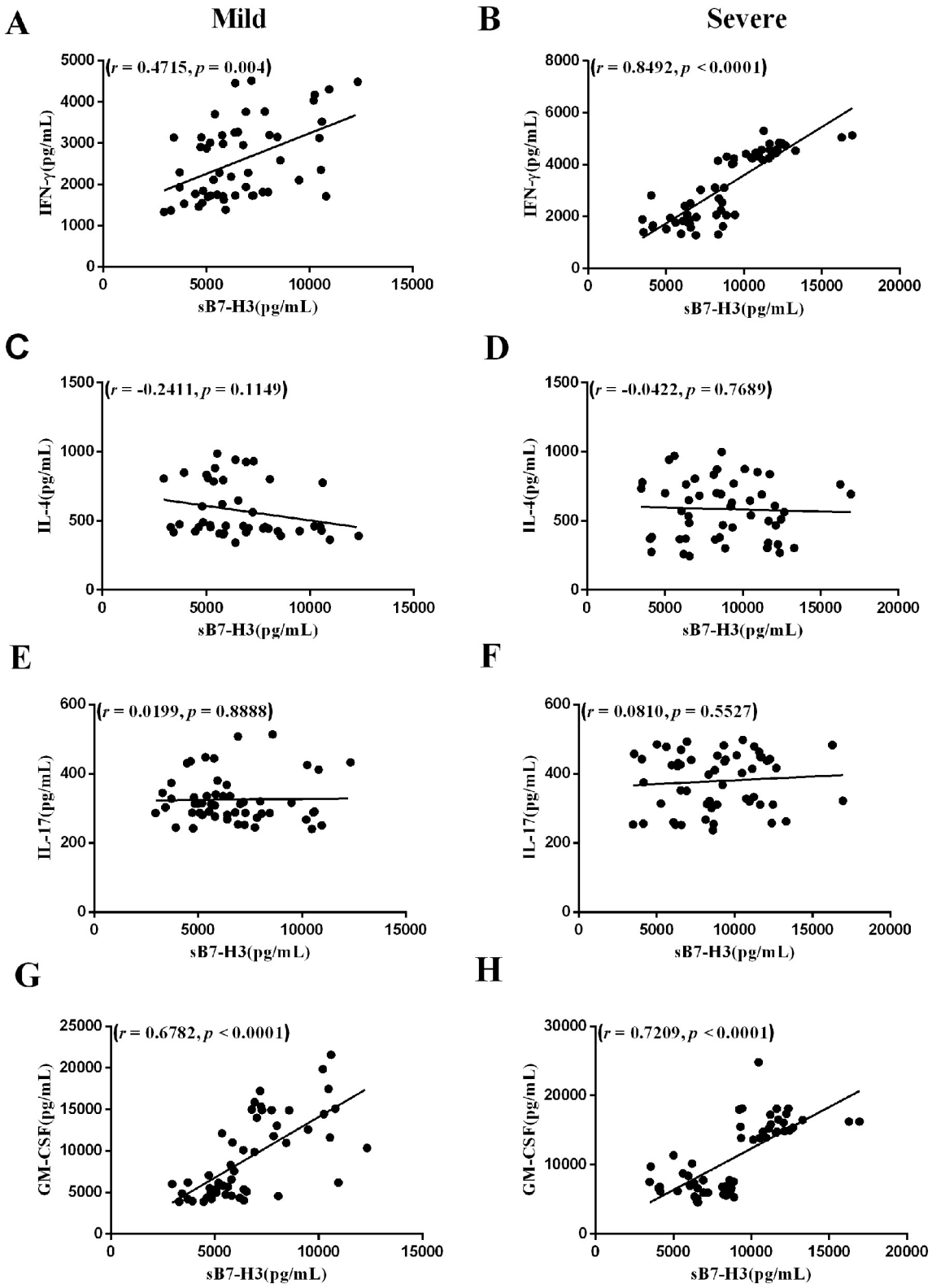

Fig. 3. Correlation analyses of between soluble B7-H3 and related inflammatory cytokines in pediatric patients with mild (n = 52) and severe (n = 56) Mycoplasma pneumoniae pneumonia (MPP). Associations were found between plasma soluble B7-H3 levels and IFN-γ (mild: $r = 0.472$, $p = 0.004$; severe: $r = 0.849$, $p < 0.001$) and GM-CSF (mild: $r = 0.678$, $p < 0.001$; severe: $r = 0.721$, $p < 0.001$) in pediatric patients with mild and severe MMP.

which are critical for host defense [26]. Similar pattern of plasma level changes was found in IFN-γ among mild, severe MPP and control subjects as sB7-H3. However, the severity prediction ability of IFN-γ (AUC = 0.6567, $p = 0.0063$) was slightly weaker than sB7-H3. Moreover, a positive correlation was found between plasma sB7-H3 and IFN-γ (mild: $r = 0.4715$, $p = 0.004$; severe: $r = 0.8492$, $p < 0.001$) in mild or severe MPP patients. However, the efficacy of IFN-γ on treatment efficacy monitoring was not obvious based on the plasma level

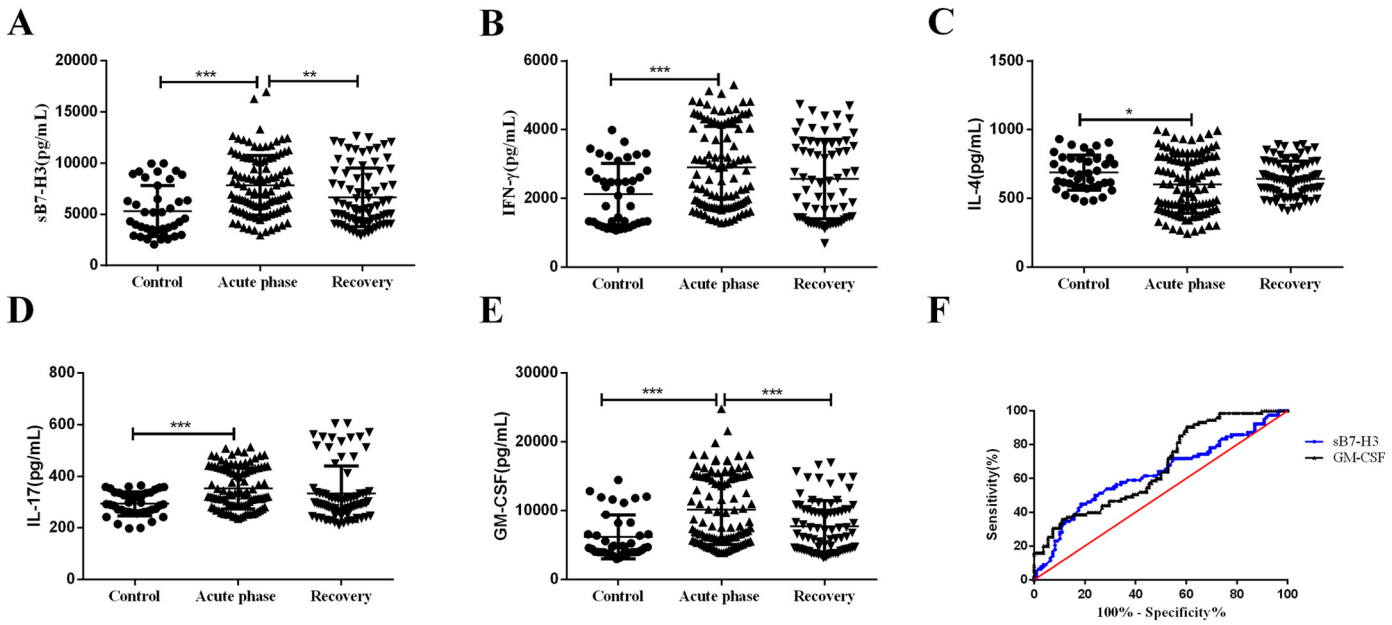

Fig. 4. Plasma level of soluble B7-H3 and related inflammatory cytokines in control (n = 40) and pediatric patients with Mycoplasma pneumoniae pneumonia (MPP) at acute phase (n = 108) and recovery stage (n = 78). sB7-H3 (A), IFN-γ (B), IL-4 (C), IL-17 (D), and GM-CSF (E) were measured and compared as described in the Methods. Among these indexes, only soluble B7-H3 and GM-CSF showed significant comparison effects between controls, acute phase and recovery stage. Therefore, ROC curves were constructed for differentiation the efficacy of sB7-H3 and GM-CSF for prognosis prediction in MPP patients (F).

comparison between acute and recovery phase MPP patients.

IL-17, a recently discovered cytokine mainly secreted by Th17 cells, is an important inflammatory mediator in the development of asthma [27,28] and protection from pneumococcal colonization [29,30]. Similar pattern of plasma level changes was found in IL-17 among mild, severe MPP and control subjects. However, the severity prediction ability of IL-17 (AUC = 0.6877, p = 0.0008) was slightly weaker than sB7-H3. However, no correlation was found between plasma sB7-H3 levels in mild or severe MPP patients and IL17. Moreover, the efficacy of IL-17 on treatment efficacy monitoring was also not significant based on the plasma level comparison between acute and recovery phase MPP patients.

According to previous studies [31,32], GM-CSF is cytokine secreted by T cells enhanced IL-6 and IL-1β production by macrophages, which in turn enhanced differentiation of IL-17A– and/or GM-CSF–producing T cells and infiltration of neutrophils into lung. Here, we also observed the changes of GM-CSF in MPP. Similar pattern of plasma level changes was found in GM-CSF among mild, severe MPP and control subjects as sB7-H3. Although slightly lower the severity prediction ability of GM-CSF (AUC = 0.6546, p = 0.0059) than sB7-H3, a positive correlation was found between plasma sB7-H3 levels and GM-CSF levels (mild: r = 0.6782, $p < 0.001$; severe: r = 0.7209, $p < 0.001$) in mild or severe MPP patients. Moreover, the efficacy of GM-CSF on treatment efficacy monitoring was comparable to sB7-H3 based on their plasma level in acute and recovery phase MPP patients.

In addition, we did not observe the efficacy of IL-4, an anti-inflammatory cytokine secreted by Th2 cells [33], in MPP severity prediction or treatment monitoring. Medjo et al. previous showed that no significant difference in serum level of IL-4 between children with MPP and those with non-MPP, which is consistent with our results.

Macrolides have been traditionally used as first-line antibiotics in children with MPP. However, the prevalence of macrolide-resistant MPP has rapidly increased since 2000, especially in Asian countries, recently reaching up to 80%–90% [34]. According to previous studies, early treatment with corticosteroids was associated with a better outcome in patients with severe MP [35,36]. We here evaluated the sB7-H3 level at pre- and post-treatment time points in MPP patients treated with corticosteroids, and the results showed that decreased level of sB7-H3 after corticosteroids application in severe MPP patients. These results implicated the sensitivity of sB7-H3 in treatment efficacy monitoring during corticosteroids application.

Since the plasma level of molecules only provide indirect evidence of the involvement of B7-H3 in MPP, and failure to obtain BALF from MPP patients initiated the necessity to establish a mouse model of MPP to examine the B7-H3 level in vivo. By intranasal instillation of LAMPs (important pathogenic structure for MP) [37] and dexamethasone treatment, we tried to recapitulate the disease condition in human and explore the role of B7-H3. The results showed that decreased levels of sB7-H3 were found in mice from Dexamethasone group compared to LAMP group, and a significant difference was found at 48 h. Direct involvement of B7-H3 during MPP and corticosteroids treatment was confirmed.

There are also some limitations in current study. Firstly, the patient number in mild and severity MPP analyses was relatively small. Second, the change of B7-H3 was only examined in 2 time points. Third, due to lacking of knockout mice, the exact effects of B7-H3 during MPP have not been evaluated. Therefore, further study with a large cohort of patients and in vivo gene-function should be performed to confirm the results obtained here.

In conclusion, we demonstrated that significantly changes of plasma B7-H3, IFN-γ, IL-17, and GM-CSF levels in children subjected to MPP after glucocorticoid treatment. Moreover, high B7-H3/IFN-γ/GM-CSF and low IL-17 levels in plasma may suggest severe MPP in children. These results suggest that sB7-H3, IFN-γ, IL-17, and GM-CSF could be used as a valuable biomarker to distinguish severe MPP from mild MPP but also a treatment efficacy predictor for children under MPP.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.05.014.

Abbreviations

ARDS	acute respiratory distress syndrome
BALF	bronchoalveolar lavage fluid
CAP	community acquired pneumonia
ELISA	enzyme linked immunosorbent assay
H&E	hematoxylin and eosin

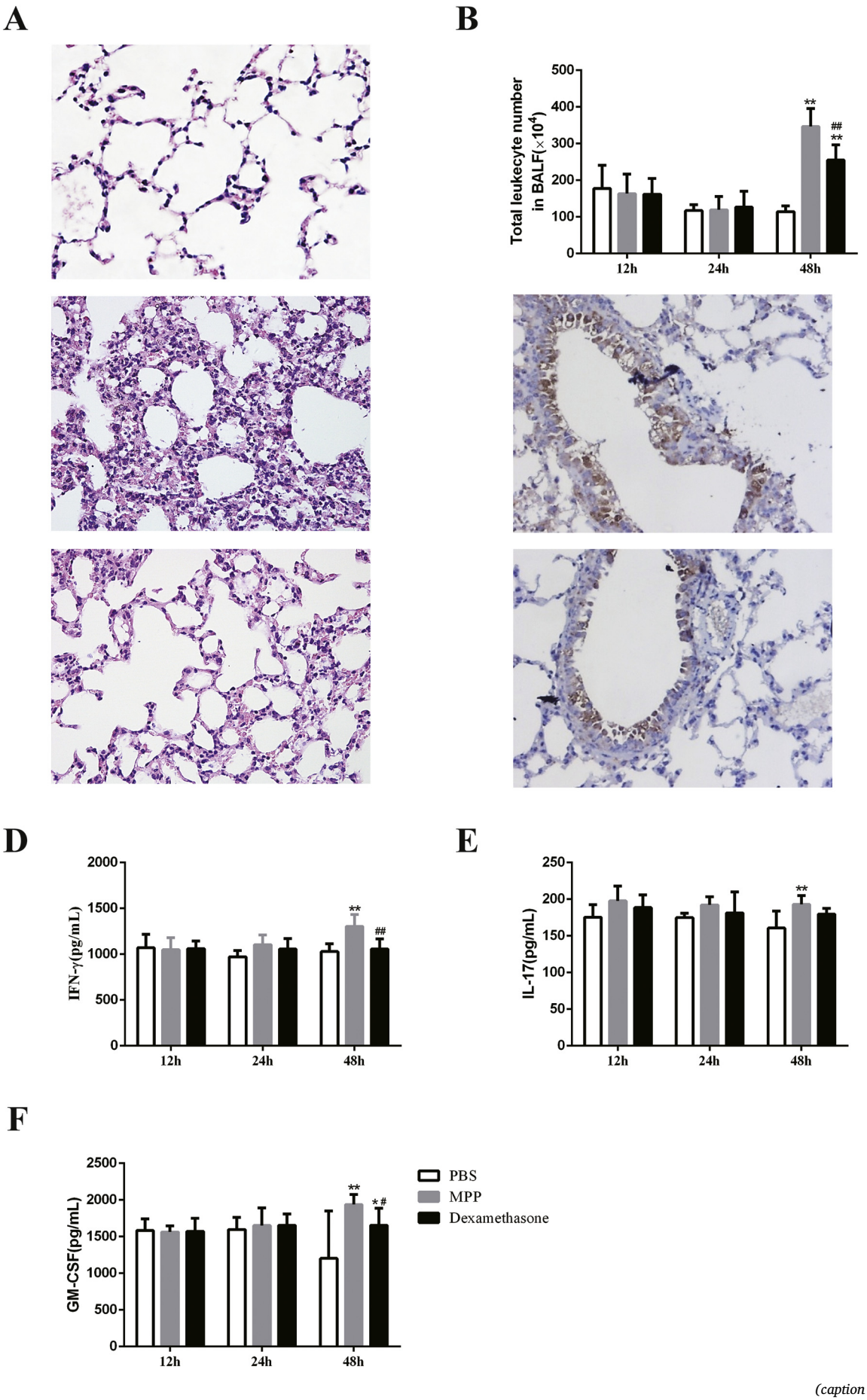

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Fig. 5. Downregulated soluble B7-H3 was observed in Dexamethasone treated *Mycoplasma pneumoniae* pneumonia (MPP) model induced by lipid associated membrane proteins (LAMPs). The representative images of hematoxylin and eosin stained sections of lung tissues of mice from Control group (A, up), MPP group (A, middle) and Dexamethasone group (A, down). B. BALF leukocyte counts in mice from Control group, MPP group and Dexamethasone group at 12 h, 24 h, and 48 h. Representative images of immunohistochemical stained sections of lung tissues in mice from MPP group (C, up) and Dexamethasone group (C, down). The levels of IFN-γ (D), IL-17 (E), and GM-CSF (F) in BALF of mice from Control group, MPP group and Dexamethasone group at 12 h, 24 h, and 48 h after LAMPs inhalation. $^{*}p < 0.05$, $^{**}p < 0.01$ versus Control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus MPP group.

LAMPs	lipid associated membrane proteins
MP	*Mycoplasma pneumoniae*
MPP	*Mycoplasma pneumoniae* pneumonia
PCR	polymerase chain reaction
PPLO	Pleuropneumonia-Like Organisms
ROS	NO and reactive oxygen species
sB7-H3	soluble B7-H3

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Ethical approval

The study was approved by the Ethics Committee of first affiliated hospital of Soochow University. All participants of this study had been informed and signed the consent for participation in this study.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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