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Original Article

Clinical value of monitoring cytokine levels for assessing the severity of mycoplasma pneumoniae pneumonia in children

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Abstract: Background: To investigate the clinical relevance of cytokine levels in assessment of the severity of mycoplasma pneumoniae pneumonia (MPP) in children. Methods: A retrospective study was conducted on 150 pediatric cases of MPP admitted to a local hospital in China from November 1, 2022 to October 31, 2023. These MPP cases were divided into mild (n=100) and severe (n=50) groups according to the severity of the disease. Cytokine levels, including Interferon-γ (IFN-γ), Tumor Necrosis Factor-α (TNF-α), C-reactive protein (CRP), Interleukin-6 (IL-6), Interleukin-2 (IL-2), and D-Dimer (D-D), were compared between the two groups. The diagnostic efficacy of each cytokine in assessing the severity of MPP was analyzed through Receiver Operating Characteristic (ROC) curves, and correlation between cytokine levels and disease severity was assessed using Pearson's correlation coefficient. Results: The IL-2 level was significantly lower, while TNF-α, IL-6, and IFN-γ levels were significantly higher in the severe group compared to the mild group (all P<0.05). TNF-α, IFN-γ, IL-2, IL-6, CRP, and D-D were identified as factors influencing the severity of MPP (all P<0.05). The ROC curve analysis showed that the areas under the curve (AUCs) of TNF-α, IL-2, IL-6, IFN-γ, CRP, and D-D were 0.864, 0.692, 0.874, 0.949, 0.814, and 0.691, respectively (all P<0.001), indicating their diagnostic value in assessing the severity of MPP. There exists a positive correlation between IL-2 and the percentage of normal lung density on Computed Tomography (CT) scan (P<0.05), while TNF-α, IL-6, IFN-γ, CRP, and D-D showed negative correlations with the percentage of normal lung density (P<0.05). Conclusion: Cytokines such as TNF-α, IL-2, IL-6, IFN-γ, CRP, and D-D are aberrantly expressed in children with MPP and are associated with the severity of the disease. These cytokines have high diagnostic value and can serve as reference indicators for clinical, especially prognostic assessment of the severity of (pediatric) MPP.

Keywords: Cytokines, mycoplasma pneumoniae pneumonia, disease severity assessment

Introduction

Mycoplasma pneumoniae (MP) is recognized as one of the important pathogens that cause MPP (Mycoplasma pneumoniae pneumonia) and respiratory diseases in children. The incidence rate of MPP is approximately 10%-40% of community-acquired pneumonia, and the incidence rate is still increasing, especially in children [1, 2]. MPP is typically diagnosed through a combination of clinical manifestation, throat swab or sputum culture, nucleic acid testing, chest X-ray, and laboratory antibody testing etc. MPP has a slow onset, with symptoms appearing 1-3 weeks after infection. Mild symptoms include fatigue, body aches, while severe symptoms include fever, persistent dry cough, chest pain, etc. If left untreated, it can progress to severe MPP, leading to complications such as lung necrosis and atelectasis, causing damage to multiple organs beyond the lungs and posing a serious threat to the lives of affected children [3]. Currently, it is believed that the onset of MPP is related to the adhesion of respiratory epithelial cells, immunopathogenic mechanisms, and invasion of Mycoplasma pneumoniae. Cytokines play an important part in the development and progression to severe MPP. Inflammatory mediators and cytokines disrupt body's immune

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response, leading to immune disturbance and disease development [4].

The helper T lymphocyte subpopulation (TH) with its cytokines play a key role in immune regulation. Among them, Interleukin-6 (IL-6), Tumor Necrosis Factor-α (TNF-α), and Interferon-γ (IFN-γ) are cytokines released by TH1 and TH2 cells, which indirectly reflect changes in TH subpopulations. They have opposing biological functions and mutually regulate and restrict each other, maintaining the body's normal immune status. They can also indirectly reflect the trend of inflammation development and play a crucial role in the anti-infection process [5]. C-reactive protein (CRP) is a non-specific marker of inflammation and tissue damage. Its level rises rapidly during inflammation and tissue damage, and the magnitude of increase is closely related to the degree of pathogenic infection [6]. Interleukin-2 (IL-2), as a core cytokine in T cell response, enhances immune activity of immune cells, including T lymphocytes, B lymphocytes, and natural killer (NK) cells. Several Studies revealed that IL-2 is aberrantly expressed in MPP and is associated with the occurrence of the disease [7]. D-Dimer (D-D) is a degraded product from fibrin, which is a relevant factor in the development of MPP. It reflects abnormal coagulation and fibrinolysis in the body. When MPP occurs, the inflammatory reaction affects the fibrinolysis system and coagulation function, leading to an increase in D-Dimer levels [8].

While significant progress has been made in understanding the pathogenesis of MPP and the role of cytokines in disease progression, current clinical practices face limitations in accurately assessing the severity of MPP, particularly in pediatric cases. The existing methods for evaluating the severity of MPP often rely on subjective clinical assessments and image findings, which may not capture the full spectrum of disease severity or provide comprehensive prognostic information. Furthermore, there is a need for identification of objective biomarkers that can reliably indicate disease severity and guide clinical decision-making. This study concentrated on exploration of specific cytokines, including IFN-γ, TNF-α, CRP, IL-6, IL-2, and D-D, in assessing the severity of MPP in children. Elucidating the association between aberrantly expressed cytokines and disease severity will help the development of more objective and accurate approaches for evaluating the severity of MPP, and thus, fill-in an existing gap in pediatric respiratory care.

Materials and methods

General information

This study retrospectively collected information from the medical records of 150 MPP children admitted to the Department of Infectious Diseases and Respiratory Medicine of Baoding Children's Hospital from November 1, 2022 to October 31, 2023. Data for this study was obtained since November 1st. All samples obtained in this study were approved by the ethics committee of the Baoding Hospital of Beijing Children's Hospital, Capital Medical University and abided by the ethical guidelines of the Declaration of Helsinki [9] (No. H-BDETKJ-SOP006-03-A/2). The study obtained written informed consent. The assessment and diagnosis of the severity of MPP for the pediatric cases were conducted using a comprehensive approach consistent with established guidelines and diagnostic criteria in China. The severity classifications were determined based on clinical presentation, physical examination findings, laboratory investigations, and radiological assessments. The diagnostic criteria including the presence of characteristic symptoms such as cough, fever, and lung consolidation signs, in addition to specific radiographic findings that distinguished severe from mild cases. The differentiation between mild and severe MPP cases was further corroborated by the duration of symptoms, response to initial treatment, and persistence of fever and worsening lung imaging despite appropriate antibiotic therapy, aligned with established clinical indicators of disease severity.

Inclusion and exclusion criteria

Inclusion criteria: (1) The respiratory symptoms and chest imaging results of the included subjects met MPP diagnostic criteria of pneumonia diagnosis and treatment guidelines [10]; (2) Complete clinical data; (3) Criteria for mild MPP diagnosis [11]: Detection of characteristic antibodies for MP in double serum samples, conversion from negative to positive for Immunoglobulin M (IgM) or an increase or decrease in Immunoglobulin G (IgG) by at least 4 times; cough, fever, and significant lung consolidation

signs on admission; X-ray findings showing more significant changes compared to physical signs; ineffective treatment with cephalosporins or penicillin; no concurrent infection with other pathogens; (4) Criteria for severe MPP diagnosis [12]: Presence of fever and worsening lung imaging after 7 days of macrolide antibiotics treatment in addition to meeting the criteria for mild MPP diagnosis. The specific criteria are as follows: confirmation can be made if at least one of the following is present: visible necrotizing pneumonia changes on chest X-ray examination, CT, or similar imaging; lobar consolidation with pleural effusion; abnormal respiratory function with extrapulmonary complications; and concomitant varying degrees of obstructive bronchitis.

Exclusion criteria: (1) Unclear pathogen diagnosis; (2) Concurrent respiratory system diseases such as pulmonary tuberculosis, bronchial asthma, and allergic rhinitis; (3) Respiratory system developmental abnormalities and malformations; (4) History of immunological diseases and recent or long-term use of glucocorticoids; (5) Abnormal coagulation function; (6) Unresolved severe pneumonia or history of surgery; (7) Presence of other serious organic diseases.

Methods

In the medical record system, investigations and statistics of patients' temperature, respiratory rate, and blood oxygen saturation were conducted. After the patients rested in a seated position for 30 minutes, 5 ml of blood was drawn from the radial artery for blood gas analysis. The analysis was performed within 10 minutes using the American IL1302 blood gas analyzer. Additionally, 5 ml of fasting venous blood was collected for a complete blood count using the fully automated blood analyzer (DxH800 blood analyzer provided by Beckman Coulter), including Procalcitonin, ESR, WBC, and Neutrophils. Patient throat swabs were taken for bacterial strain isolation and testing for antibiotic resistance. The bacterial strains were isolated and tested for antibiotic resistance using culture medium, M-H culture medium, and antibiotic paper purchased from Hangzhou Tianhe Biological Products Co., Ltd. The types of antibiotics tested included: Azithromycin, Clarithromycin, Levofloxacin, Doxycycline, Moxifloxacin.

Cytokine indicator detection: Within 24 hours of admission, fasting peripheral blood samples of 4 ml were collected and centrifuged at 3000 rpm, with serum separated. Supernatant was then stored at -80°C for further testing. The detection indicators included TNF-α, IL-2, IL-6, IFN-γ, CRP, and D-D, measured with enzyme-linked immunosorbent assay (ELISA). The kits were purchased from America abcam. ELISA kit: TNF-α (ab181421), IL-2 (ab283543), IL-6 (ab178013), IFN-γ (ab174443), CRP (ab-260058), and D-D (ab315310). Procedures were strictly followed according to the instructions. The procedure is shortened here: (1) all reagents and standards were arranged as instructed; (2) 90 μl of sample was added to each well and incubated for 1.5 h at room temperature; (3) 90 μl of the antibody was added to each well and incubated for 2 h; (4) 90 μl of the Streptavidin solution was added and incubated for 1 h; (5) 100 μl of substrate tetramethylbenzidine (TMB) was added to each well and incubated for 40 min; (6) 50 μl of Stop Solution was added to each well, and plates were immediately read at 450 nm. The differences in cytokine levels between the two groups were compared, and multiple logistic regression analysis was conducted to explore the correlation between various cytokines and MPP disease. Additionally, ROC analysis was conducted to assess the value of detecting cytokines in disease evaluation.

The high-resolution CT (HRCT) protocol used in this study adhered to standardized imaging parameters to achieve optimal visualization of pulmonary parenchymal changes. The HRCT scans were performed using a 64-slice spiral CT scanner (Philips, model: Brilliance 40) with the following imaging parameters: tube voltage of 120 kVp, tube current of 150 mAs, slice thickness of 1 mm, and reconstruction interval of 1 mm. The scans were acquired without contrast administration as per the established protocol for pulmonary imaging in pediatric patients.

The interpretation of HRCT findings was conducted by two experienced radiologists specializing in pediatric pulmonary imaging. Additionally, the analysis involved evaluating lung density patterns, consolidative opacities, ground-glass opacities, and other morphological features indicative of disease severity, following standardized guidelines for pediatric

pulmonary imaging interpretation. The CT scan evaluations for determining the percentage of normal lung density were conducted by two experienced radiologists who were blinded to the clinical information and cytokine levels of the pediatric patients. Blinding the radiologists to the patients' clinical data and cytokine measurements was essential to mitigate potential bias in the assessment of lung density percentages and ensure the objective interpretation of HRCT findings. This rigorous approach aimed to minimize the influence of preexisting knowledge about the patients' MPP severity on the radiological assessments, thereby enhancing the objectivity and reliability of the lung density percentage measurements.

In this study, the levels of several key cytokines were measured to evaluate their association with the severity of MPP in children. The selected cytokines included Interferon-γ (IFN-γ), Tumor Necrosis Factor-α (TNF-α), C-reactive protein (CRP), Interleukin-6 (IL-6), Interleukin-2 (IL-2), and D-Dimer (D-D). These cytokines were chosen based on their known roles in immune regulation, inflammation, and tissue damage. IFN-γ and TNF-α, produced by TH1 cells, reflect changes in TH subpopulations and play crucial roles in the anti-infection process. CRP is a nonspecific marker of inflammation and tissue damage, while IL-6 is a pro-inflammatory cytokine with diverse biological activities. IL-2, as a core cytokine in T cell response, enhances immune activity, and D-Dimer is a relevant factor in the development of MPP, reflecting abnormal coagulation and fibrinolysis in the body. The selection of these cytokines aimed to provide a comprehensive assessment of the immune and inflammatory responses associated with MPP, thus informing the clinical assessment of disease severity.

General reference ranges for IL-2, TNF-α, IL-6, and IFN-γ are: 100-300 pg/mL, 0-8.1 pg/mL, 1.5-5.0 pg/mL, and IFN-γ 0-20 pg/mL, respectively.

Statistical methods

SPSS 22.0 was adopted for data analysis. Count data were expressing as [n (%)] and analyzed using chi-square test. Measurement data were expressed as (Mean ± SD) and analyzed using t-test. Multiple logistic regression analysis was conducted to explore influencing factors, ROC curve analysis was performed for diagnostic value. Pearson's correlation analysis was conducted in the correlation analysis. A *P* value of less than 0.05 was considered to be statistically significant.

Results

Demographic characteristic

Among the subjects, 100 cases of mild MPP and 50 cases of severe MPP were included. The sample size of 150 pediatric cases included in this study was determined based on power calculations to ensure adequate statistical power for detecting clinically significant differences in cytokine levels between the mild and severe MPP groups. The sample size was calculated to achieve 80% power, with a significance level of 0.05, taking into account the expected effect size based on prior research and the anticipated variability in cytokine levels within the study population. In the mild group, there were 56 male patients and 44 female patients, with the mean age of (7.54 ± 2.60) years old and BMI of (20.81 ± 1.66) kg/m^2. In the severe group, there were 27 male patients and 23 female patients, with the mean age of (7.50 ± 2.73) years old and BMI of (20.40 ± 1.59) kg/m^2. No statistically significant differences in the general information such as age, gender and BMI between two groups were observed (all P>0.05), indicating comparability (**Table 1**). However, patients with severe MPP demonstrated significantly higher temperature (38.61 ± 0.77°C) compared to those with mild MPP (38.31 ± 0.74°C), (t=2.276, P=0.025). Similarly, patients with severe MPP demonstrated significantly higher respiratory rate (28.78 ± 6.24) compared to those with mild MPP (26.12 ± 6.33), (t=2.448, P=0.016). Finally, oxygen saturation were significantly lower in the severe MPP group (93.96 ± 3.99) compared to the mild MPP group (95.35 ± 3.84) (t=2.010, P=0.044).

Blood gas analysis

The blood gas analysis revealed differences between mild and severe MPP cases (**Figure 1**). Specifically, patients with severe MPP demonstrated significantly lower pO_2 levels [(77.28 ± 8.31 mmHg vs 80.63 ± 8.27 mmHg), (t=2.328, P=0.022)], lower pH levels [(7.44 ± 0.05) vs (7.46 ± 0.05), (t=2.448, P=0.016)], lower HCO_3^- levels [(22.61 ± 3.77 mEq/L) vs (23.87 ± 3.24

Table 1. Comparison of general data, clinical symptoms and signs between mild and severe mycoplasma pneumoniae pneumonia groups

Parameters	Mild (n=100)	Severe (n=50)	t/χ^2	*P* Value
Age (years)	7.54 ± 2.6	7.5 ± 2.73	0.067	0.947
Gender (M/F)	56 (56%)/44 (44%)	27 (54%)/23 (46%)	0.003	0.954
BMI (kg/m^2)	20.81 ± 1.66	20.4 ± 1.59	1.469	0.145
Fever (°C)	38.31 ± 0.74	38.61 ± 0.77	2.276	0.025
Respiratory Rate	26.12 ± 6.33	28.78 ± 6.24	2.448	0.016
Oxygen Saturation	95.35 ± 3.84	93.96 ± 3.99	2.040	0.044

Note: BMI, Body mass index.

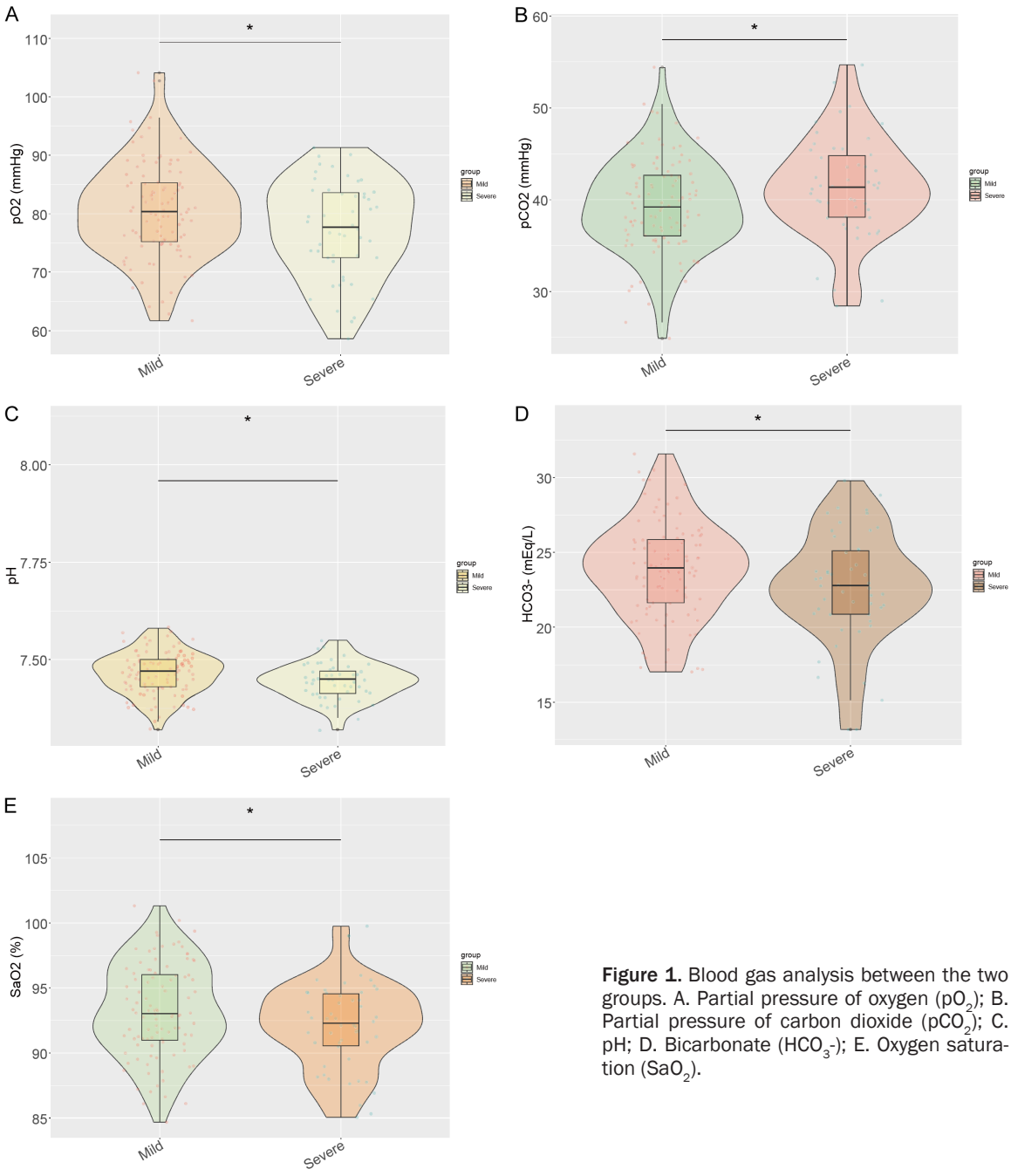

Figure 1. Blood gas analysis between the two groups. A. Partial pressure of oxygen (pO_2); B. Partial pressure of carbon dioxide (pCO_2); C. pH; D. Bicarbonate (HCO_3-); E. Oxygen saturation (SaO_2).

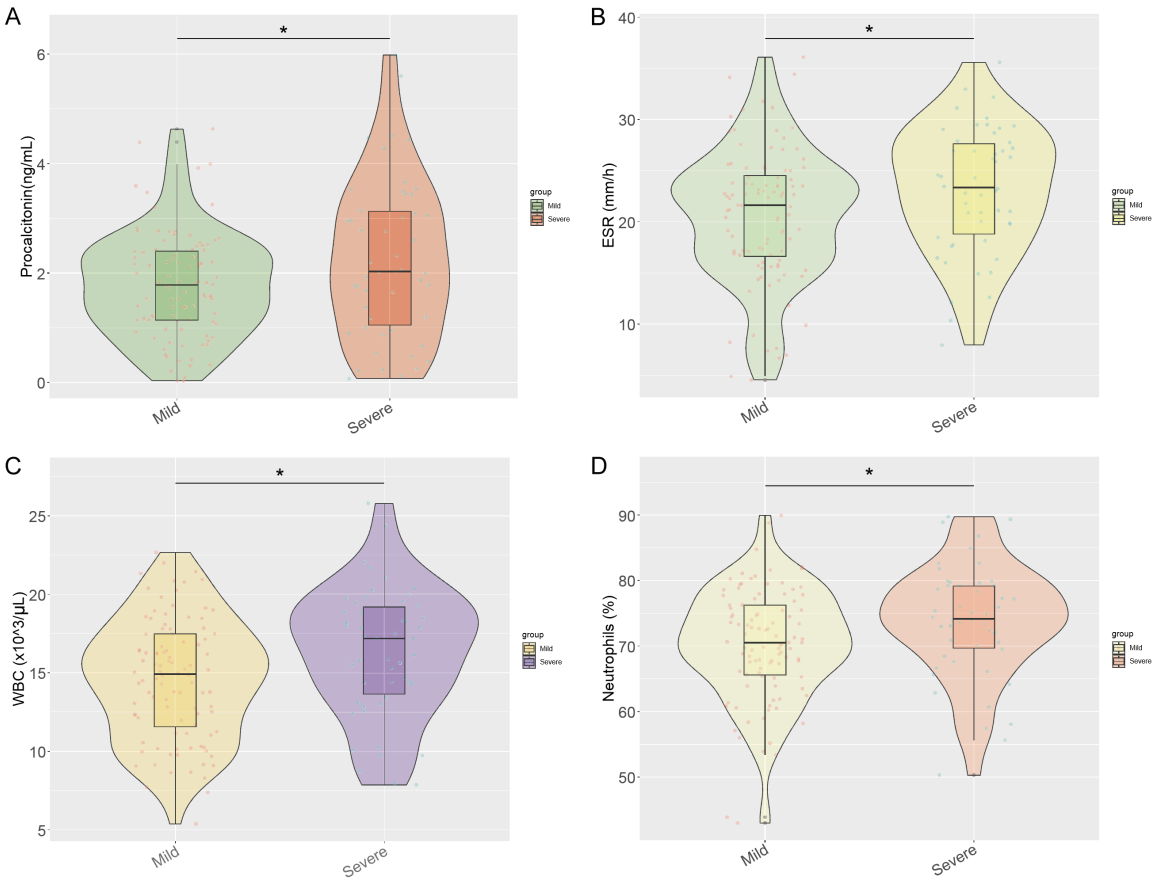

Figure 2. Inflammatory markers in mild vs severe mycoplasma pneumoniae pneumonia. A. Procalcitonin; B. Erythrocyte sedimentation rate (ESR) levels; C. White blood cell (WBC) counts; D. Neutrophils.

mEq/L), (t=2.010, P=0.048)], and lower SaO_2 levels [(92.03 ± 3.26%) vs (93.23 ± 3.59%), (t=2.051, P=0.043)] compared to those with mild MPP. Conversely, pCO_2 levels were significantly higher in the severe MPP group [(41.32 ± 5.53 mmHg) vs (39.38 ± 5.15 mmHg), (t=2.076, P=0.041)] compared to the mild MPP group.

Inflammatory markers

In the comparison of inflammatory markers between mild and severe MPP cases, several significant differences were observed (**Figure 2**). The severe group displayed significantly higher levels of procalcitonin [(2.24 ± 1.45 ng/mL) vs (1.78 ± 0.96 ng/mL), (t=2.038, P=0.045)], higher levels of ESR [(23.07 ± 6.18 mm/h) vs (20.62 ± 6.57 mm/h), (t=2.241, P=0.027)], higher WBC counts [(16.31 ± 4.11 × 10^3/μL) vs (14.59 ± 3.83 × 10^3/μL), (t=2.472, P=0.015)], and higher percentage of neutrophils [(73.78 ± 8.62%) vs (70.25 ± 8.51%), (t=2.373, P=0.020)] than the mild MPP group.

Antibiotic resistance patterns

In evaluating antibiotic resistance patterns in mild versus severe MPP cases, the analysis unveiled some noteworthy trends (**Table 2**). While the differences in antibiotic resistance percentages for azithromycin, clarithromycin, levofloxacin, doxycycline, and moxifloxacin did not reach statistical significance (all P>0.05), there were notable higher resistance percentages in the severe group for all antibiotics. For azithromycin, there were 13.00% of mild cases and 26.00% of severe cases exhibiting resistance (χ^2=3.077, P=0.079). Similarly, clarithromycin resistance was observed in 10.00% of mild cases and 20.00% of severe cases (χ^2=2.084, P=0.149). In addition, resistance percentages for levofloxacin, doxycycline, and moxifloxacin showed a similar pattern between

Table 2. Antibiotic resistance patterns in mild and severe mycoplasma pneumoniae pneumonia

Antibiotic	Mild (n=100)	Severe (n=50)	t/χ^2	*p*-value
Azithromycin (%)	13 (13.00%)	13 (26.00%)	3.077	0.079
Clarithromycin (%)	10 (10.00%)	10 (20.00%)	2.084	0.149
Levofloxacin (%)	7 (7.00%)	9 (18.00%)	3.157	0.076
Doxycycline (%)	5 (5.00%)	7 (14.00%)	2.548	0.110
Moxifloxacin (%)	6 (6.00%)	8 (16.00%)	2.846	0.092

Table 3. Comparison of cytokine levels between the two groups

Factors	Mild (n=100)	Severe (n=50)	t	P
IL-2 (pg/mL)	70.17 ± 11.97	47.91 ± 8.87	11.645	<0.001
IL-6 (pg/mL)	47.95 ± 4.52	62.17 ± 11.14	11.096	<0.001
TNF-α (pg/mL)	73.29 ± 8.78	96.91 ± 18.57	10.592	<0.001
IFN-γ (pg/mL)	199.02 ± 37.70	298.99 ± 48.54	13.873	<0.001
CRP (pg/mL)	37.45 ± 7.91	48.93 ± 9.19	7.928	<0.001
D-D (pg/mL)	0.81 ± 0.18	1.30 ± 0.30	12.263	<0.001

Notes: IFN-γ, Interferon-γ; TNF-α, Tumor Necrosis Factor-α; CRP, C-reactive protein; IL-6, Interleukin-6; IL-2, Interleukin-2; D-D, D-Dimer.

Table 4. Logistic regression analysis on influencing factors of disease progression

Factors	β	S.E.	Wals	OR	P	Exp (β)	95% CI	
							Upper	Lower
IL-2	0.232	0.043	28.994	1.236	<0.001	0.793	0.729	0.863
IL-6	0.263	0.049	28.811	1.630	<0.001	1.301	1.182	1.432
TNF-α	0.136	0.024	31..050	1.834	<0.001	1.146	1.092	1.203
IFN-γ	0.070	0.014	23.396	1.810	<0.001	1.072	1.042	1.103
CRP	0.195	0.054	13.095	1.130	<0.001	1.215	1.093	1.350
D-D	2.238	0.607	13.618	1.512	<0.001	9.377	2.856	30.785

Notes: IFN-γ, Interferon-γ; TNF-α, Tumor Necrosis Factor-α; CRP, C-reactive protein; IL-6, Interleukin-6; IL-2, Interleukin-2; D-D, D-Dimer.

the mild and severe groups, although without statistical significance (P>0.05).

Cytokine levels

In the severe MPP group, the mean level of IL-2 was 47.91 ± 8.87 pg/mL and that in the mild group was 70.17 ± 11.97 pg/mL (P<0.001). Conversely, TNF-α level [96.91 ± 18.57 pg/mL vs 73.29 ± 8.78 pg/mL, (P< 0.001)], IL-6 level [62.17 ± 11.14 pg/mL vs 47.95 ± 4.52 pg/mL, (P<0.001)], IFN-γ level [298.99 ± 48.54 pg/mL vs 199.02 ± 37.70 pg/mL, (P<0.001)], CRP level [48.93 ± 9.19 pg/mL vs 37.45 ± 7.91 pg/mL, (P<0.001)], and D-D level [1.30 ± 0.30 pg/mL vs 0.81 ± 0.18 pg/mL, (P<0.001)] were significantly higher in the severe MPP group compared to the mild MPP group, as demonstrated in **Table 3**. These provide a more precise understanding of the associations between cytokine expression and MPP severity.

Logistic regression analysis on factors influencing progression of MPP

The logistic regression analysis was conducted to identify the factors impacting the disease progression in pediatric patients, with severe MPP as the dependent variable and the statistically significant cytokines as the independent variables. The results indicated that TNF-α, IL-2, IL-6, IFN-γ, CRP, and D-D were significant factors influencing the progression of the disease in MPP patients (P<0.05), as shown in **Table 4**.

Value of cytokine assessment in evaluating the severity of MPP

The diagnostic value of cytokine assessment for the severity of MPP was evaluated using ROC analysis. The results revealed that the AUCs for TNF-α, IL-2, IL-6, IFN-γ, CRP, and D-D were 0.923, 0.874, 0.87, 0.951, 0.824 and 0.916 with high specificity and sensitivity, indicating good evaluation effectiveness, as illustrated in **Figure 3**.

Correlation analysis of cytokines with CT lung normal density volume percentage

The CT lung normal density volume percentage in the mild group was (73.05 ± 8.85) × 10^{-2}, which was significantly larger than (49.14 ± 6.82) × 10^{-2} in the severe group (t=16.766, P<0.05). The CT images of the mild and severe groups are shown in **Figures 4** and **5**.

The correlation analysis revealed that IL-2 was positively correlated with the CT lung normal density volume percentage (P<0.05), while IFN-γ, IL-6, TNF-α, CRP, and D-D were negatively correlated with the CT lung normal density volume percentage (all P<0.05), as demonstrated in **Table 5**.

Discussion

Mycoplasma pneumonia (MPP) is a frequent respiratory disease with an increasing incidence and a significant trend towards affecting younger age groups [13]. The pathogenesis of MPP is complex, with immune mechanisms playing a key role in the pathological changes of disease. It is currently believed that T cell subset dysregulation, humoral immunity, and cellular immunity are involved in the development of MPP. Among them, cell-mediate immune reactions, especially release of a large number of cytokines, play a key role in clinical progression as well as pathological features in MPP infection [14]. CRP is an acute-phase protein, activating complement and enhances phagocytosis, clearing invading pathogens and damaged, necrotic, as well as apoptotic tissue cells. It has been used as an important reference index for differential diagnosis of viral and bacterial pneumonia [15, 16].

D-D is a degradation product of fibrin and is commonly used in coagulation function tests. As an important factor in the coagulation and fibrinolysis abnormalities of the body, the inflammatory response in MPP patients can significantly affect the fibrinolysis system and coagulation function, leading to abnormally elevated levelso f serum D-D [17]. The results in the current study showed that CRP and D-D levels in severe group were significantly higher than those in mild group. The reason for this may be that Mycoplasma pneumoniae directly damages cell membranes, exposing acetylcholine phosphate molecules and attachment points for CRP. Through IL-6 and other mediators, this information is transmitted to the liver, resulting in the production of a large amount of CRP. Therefore, CRP is also a sensitive indicator of tissue damage caused by Mycoplasma pneumoniae (MP). Severe MPP can activate macrophages, stimulate the secretion of IL-1, TNF, and other inflammatory factors, causing adhesion of pathogens, damage to vascular endothelial function, and resulting in concentrated and aggregated phenomena in the body, leading to diffuse intravascular coagulation and microthrombosis, which in turn causes an increased D-D levels. Previous study has also showed the role of D-D levels in the clinical setting of MPP [18]. Both CRP and D-D can be used as reference indicators for assessing the severity of MPP [19].

IL-2, as a cell growth factor in the immune system, regulates activity of WBC. It acts on both secretory and adjacent cells through autocrine and paracrine mechanisms, exerting effects on various cells. Additionally, IL-2 directly affects B cells and promotes T cell growth, thereby regulating immune responses. Study [20] has shown that the production of antigen-specific Th1 cells is associated with the immunopathological processes of various infectious diseases, and IL-2 promotes differentiation from Th0 to Th1. Our study showed that IL-2 level in severe group were lower than those in mild group, suggesting the involvement of IL-2 in the progression of MPP. Research conducted by Yu Ming et al. [21] has shown that the levels of IL-2 in acute and severe MPP patients were reduced, indicating a decline in immune function during this period. The possible reason for this is that after infection with MP, helper Th1 cells become dominant, leading to release of pro-inflammatory factors and activation of alveolar macrophages and neutro-

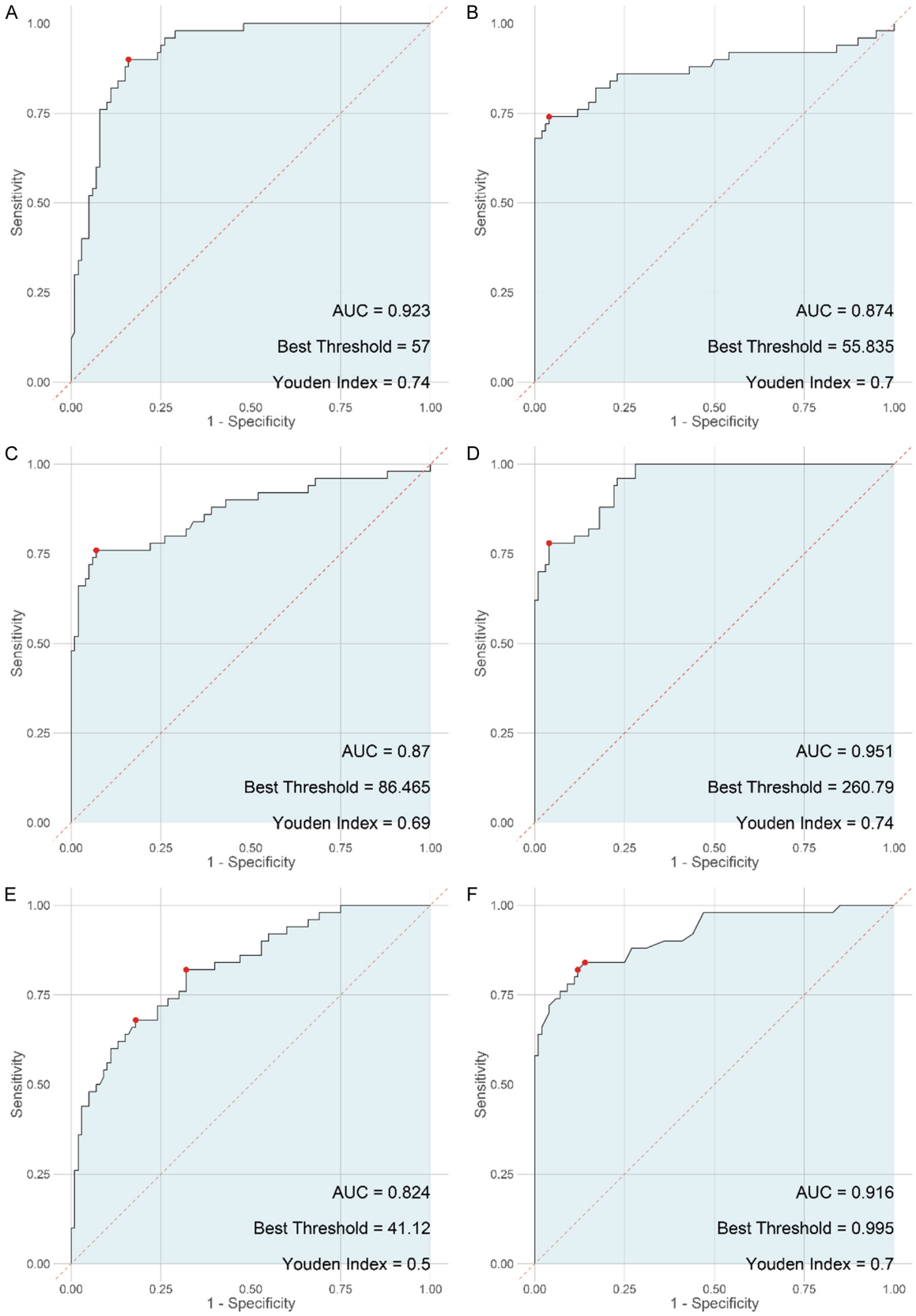

Figure 3. ROC curve for MPP severity evaluation by various cytokines. A. Interleukin-2 (IL-2); B. Interleukin-6 (IL-6); C. Tumor Necrosis Factor-α (TNF-α); D. Interferon-γ (IFN-γ); E. C-reactive protein (CRP); F. D-Dimer (D-D).

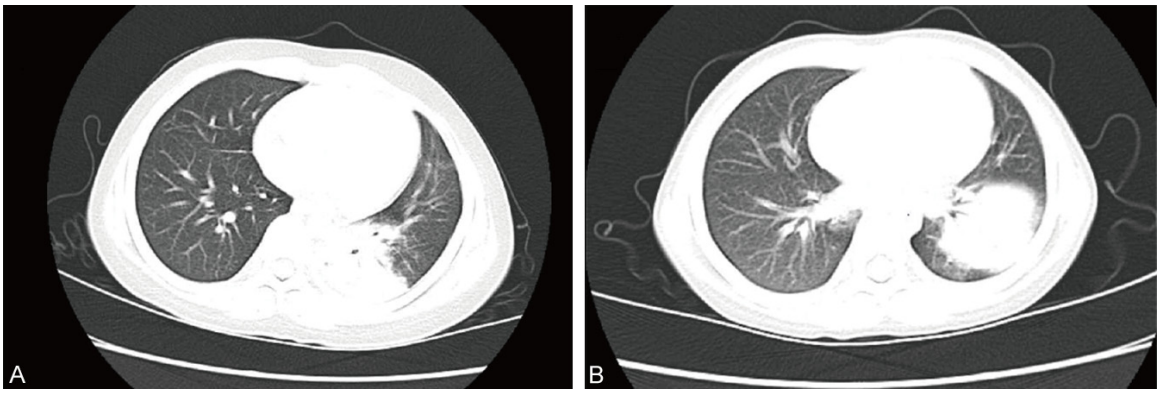

Figure 4. The CT images of 2 patients in the mild group. A. In the left inferior lobe of the anterior basal segment, there were wedge-shaped solid shadows with air bronchial signs. In the remaining left lung, there were multiple patchy ground glass density shadows distributed along the bronchial vascular bundles, and the bronchial tube wall was thickened; B. The dorsal segment of the lower lobe of the left lung became solid, and the lesions were distributed along the right interlobar pleura.

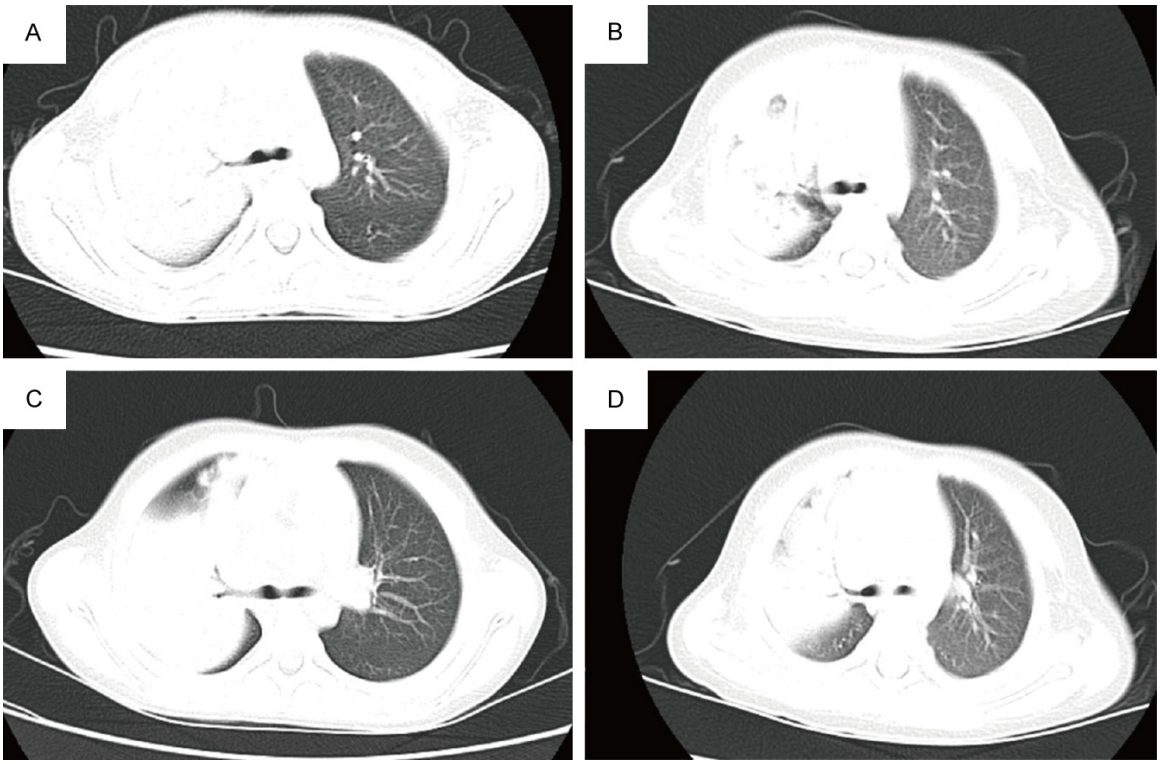

Figure 5. The CT images of 4 patients in the severe groups. A. Thickening and blurring of lung texture; B. Lung consolidation; C. A cloudy, high-density shadow of the right lung along the bronchus, with ground-glass changes; D. Lung consolidation.

phils. These cells penetrate the capillary wall and reach the site of pathogen invasion, resulting in increased local tissue inflammation and affecting the progression of the disease.

IL-6 is produced by activated T cells and fibroblasts. It has multiple biological activities, including immune regulation, and acts as a pro-inflammatory factor, catalyzing and amplifying inflammatory responses [22]. The level of IL-6 can reflect the severity of cellular and tissue damage and is a biomarker for the diagnosis of clinical acute and chronic inflammation. The research conducted by Zhang et al. [23] showed

Table 5. Correlation between cytokines and CT lung normal density volume percentage

Factors	CT lung normal density volume percentage	
	r	P
IL-2	0.549	<0.001
IL-6	-0.587	<0.001
TNF-α	-0.585	<0.001
IFN-γ	-0.602	<0.001
CRP	-0.424	<0.001
D-D	-0.606	<0.001

Notes: IFN-γ, Interferon-γ; TNF-α, Tumor Necrosis Factor-α; CRP, C-reactive protein; IL-6, Interleukin-6; IL-2, Interleukin-2; D-D, D-Dimer.

that during the acute phase of MPP, T cell differentiation was promoted, leading to the release of inflammatory cytokines and an increase in serum IL-6 levels. During the recovery period, the concentration of IL-6 decreases, and there is a correlation between IL-6 and the severity of MPP. The results in our study showed that the levels of IL-6 in severe group were higher, confirming the relationship between IL-6 levels and MPP progression in children.

TNF-α is produced by macrophages and monocytes, which are closely related to infection and inflammatory responses. TNF-α has a wide range of biological activities and plays a key role in synthesis and inflammatory mediators release, complement activation, and neutrophil aggregation. It is an important pro-inflammatory factor [24, 25]. In our study, levels of TNF-α in the severe group were higher, suggesting a close correlation between TNF-α levels and the severity of MPP.

IFN-γ is important in the early defense response to MP infection. It is produced by natural killer cells, activated T cells and natural killer T cells. IFN-γ promotes development of Th1 cells as well as activation and proliferation of Th2 cells. It also regulates major histocompatibility complex (MHC) class I and II molecules expression on monocytes, macrophages, which participate in the processes of antigen presentation and specific immune recognition, thereby eliminating MP. Our study revealed that IFN-γ levels in severe group were higher, which was similar to the findings as reported. [26], suggesting that IFN-γ not only participates in MPP pathogenesis, but also correlates with the disease severity.

Logistic regression analysis in this study showed that TNF-α, IL-2, IL-6, IFN-γ, CRP, and D-D are factors influencing the severity of MPP. ROC analysis showed that the AUCs of TNF-α, IL-2, IL-6, IFN-γ, CRP, and D-D were 0.864, 0.692, 0.874, 0.949, 0.814, and 0.691, respectively, for evaluating the severity of MPP in children, indicating that these cytokines have important diagnostic value in assessing the severity of the disease and can serve as reference indicators for clinical evaluation. Furthermore, this study showed that the percentage of normal lung density volume on CT scans in severe group was significantly lower. Correlation analysis indicated a positive relationship between IL-2 and percentage of normal lung density volume, and a negative relationship between IL-6, TNF-α, IFN-γ, CRP, D-D, and the percentage of normal lung density volume. This further confirms the role of cytokine levels in the progression of MPP and their correlation with disease severity, indicating the importance of measuring cytokine levels in evaluating the disease.

The selection of the HRCT parameter aligns with the growing recognition of quantitative imaging techniques as valuable tools for assessing pulmonary pathologies, including pneumonia. Quantitative analysis of lung density on HRCT can provide nuanced insights into the extent of parenchymal involvement, consolidation, and ground-glass opacities, offering a more comprehensive depiction of disease severity beyond traditional qualitative assessments. In the context of MPP, the percentage of normal lung density volume serves as a quantifiable metric reflecting the degree of lung parenchyma preservation and the extent of inflammatory changes, thereby adding an objective dimension to the clinical evaluation of MPP severity. The integration of HRCT parameters in parallel with cytokine monitoring enriches the multidimensional approach to assessment of MPP severity, offering a comprehensive framework for prognostic assessment and treatment stratification. Future research could delve into the correlations between HRCT parameters, cytokine expression, and clinical outcomes to elucidate their collective value in refining risk stratification and optimizing therapeutic decision-making in pediatric MPP.

According to literature, previous studies by Chen et al. [27] have reported similar patterns

of cytokine dysregulation in MPP, with elevated levels of TNF-α, IL-6, and IFN-γ correlating with disease severity. Furthermore, our findings regarding the decreased levels of IL-2 in severe MPP cases are consistent with the observations by Wang et al. [28], which highlighted the downregulation of IL-2 in pediatric respiratory infections associated with severe disease. By drawing parallels with these established studies, our research underscores the reproducibility and clinical significance of the observed cytokine alterations in MPP. Moreover, the identification of CRP and D-D as factors influencing disease severity is consistent with the recognized role of these markers in reflecting the extent of inflammation and coagulation abnormalities in respiratory infections. Overall, the parallels drawn from the existing literature are consistent with our findings, which contribute to a deeper understanding of the immunological mechanisms driving MPP pathogenesis and progression.

We emphasize the clinical relevance of cytokine levels as monitoring indicators and their potential impact on refining management approaches for pediatric MPP. These findings are of significant clinical relevance, offering valuable insights into the subtle facets of pediatric MPP assessment. By elucidating the relationships between various cytokines, including IFN-γ, TNF-α, CRP, IL-6, IL-2, and D-D, and the severity of patients' conditions, this analysis provides practical guidance for clinicians involved in managing pediatric MPP. The strong correlations observed between cytokines and disease severity underscore the value of cytokines in guiding treatment plans, resource allocation, and risk stratification.

While the present study provides valuable insights into the relationship between cytokine expression and the severity of MPP in children, several potential limitations should be acknowledged. Firstly, the small sample size may introduce some statistical bias, limiting the generalizability of the findings. Future investigations with larger, more diverse cohorts would help to validate and broaden the applicability of the observed cytokine associations with MPP severity. Secondly, the study was retrospective in design and may have introduced unconsidered confounding variables that would have had an impact on the differences in cytokine levels between the mild and severe MPP groups. Factors such as comorbidities, concurrent medications, and variations in individual immune responses could influence cytokine expression and disease severity. Additionally, the study's focus on a specific set of cytokines may overlook the potential contributions of other immune mediators that could play important roles in MPP pathogenesis. Therefore, future investigations of potential confounders are warranted to further elucidate the complex interplay between cytokine expression and MPP severity.

Conclusions

In conclusion, the findings of this study underscore the potential of cytokine monitoring as a valuable tool for assessing the severity of Mycoplasma Pneumoniae Pneumonia (MPP) in pediatric patients. The aberrant expression of TNF-α, IL-2, IL-6, IFN-γ, CRP, and D-D and their association with MPP severity highlight the diagnostic and prognostic significance of these cytokines, which provide valuable insights into disease progression and guide tailored therapeutic interventions. The potential integration of cytokine monitoring into surveillance and diagnostic algorithms could enhance the timely identification and management of MPP cases, with the ultimate goal of mitigating disease burden and improving long-term outcomes for affected children.

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Disclosure of conflict of interest

None.

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