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Clinical profiles of *Mycoplasma pneumoniae* pneumonia in children with different pleural effusion patterns: a retrospective study

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Abstract

Background The clinical significance of the presence or absence of *Mycoplasma pneumoniae* (MP) in pleural effusion in *Mycoplasma pneumoniae* pneumonia (MPP) children has not yet been elucidated. Herein, we investigated the clinical implication of pleural fluid MP positive in children with MPP.

Methods A total of 165 MPP children with pleural effusion requiring thoracocentesis were enrolled in this study. They were subsequently divided into two groups according to the presence or absence of MP in pleural effusion, namely positive group ($n=38$) and negative group ($n=127$). Information on their clinical manifestations, laboratory findings, radiological characteristics and treatment modalities was retrospectively collected from medical chart reviews.

Results The length of hospitalization (15.00 (10.75–19.25) vs. 11.00 (9.00–14.00) days, $p=0.001$) and total course of illness (23.00 (18.00–28.00) vs. 20.00 (17.00–24.00) days, $p=0.010$) were significantly longer in the positive group than in the negative group. The occurrence of pericardial effusion (23.7% vs. 7.9%, $p=0.017$), atelectasis (73.7% vs. 53.5%, $p=0.027$) and necrotizing pneumonia (23.7% vs. 7.9%, $p=0.017$) were more frequent in the positive group compared to the negative group. The levels of neutrophil percentages (82.35% (75.40%-85.78%) vs. 72.70% (64.30%-79.90%), $p<0.001$), C-reactive protein (CRP) (71.12 (37.75–139.41) vs. 31.15 (13.54–65.00) mg/L, $p<0.001$), procalcitonin (PCT) (0.65 (0.30–3.05) vs. 0.33 (0.17–1.13) ng/ml, $p=0.005$), serum lactate dehydrogenase (LDH) (799.00 (589.00–1081.50) vs. 673.00 (503.00–869.00) U/L, $p=0.009$), D-dimer (6.21 (3.37–16.11) vs. 3.32 (2.12–6.62) mg/L, $p=0.001$) on admission were significantly higher in the positive group than in the negative group. These pronounced differences significantly contributed to the identification of MPP with MP positive pleural effusion, as evidenced by the ROC curve analysis. Marked elevations in adenosine deaminase (49.25 (36.20–60.18) vs. 36.20 (28.10–46.50) U/L, $p<0.001$) and LDH levels (2298.50 (1259.75–3287.00) vs. 1199.00 (707.00–1761.00) U/L, $p<0.001$) were observed in pleural fluid of the positive group when compared to the negative group. Meanwhile, the number of patients on low molecular weight heparin (LMWH) therapy (9 (23.7%) vs. 12 (9.4%), $p=0.028$) was higher in the positive group. Multivariate logistic regression analysis revealed that D-dimer > 7.33 mg/L was significantly associated with the incidence of MP positive pleural effusion in MPP (OR=3.517).

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Conclusions The presence of MP in pleural fluid in MPP children with pleural effusion indicated a more serious clinical course. D-dimer > 7.33 mg/L was a related factor for MP positive pleural effusion in MPP. The results of the present study would help in the creation of a therapeutic plan and prediction of the clinical course of MPP in children.

Keywords Pleural effusion, *Mycoplasma pneumoniae*, *Mycoplasma pneumoniae* pneumonia, Neutrophils, D-dimer

Introduction

Mycoplasma pneumoniae (MP), a prevalent respiratory pathogen responsible for community-acquired pneumonia (CAP), accounts for 10–40% of CAP cases in children [1, 2]. MP infection was traditionally thought to be self-limited with a favorable prognosis. However, once it progresses to severe *Mycoplasma pneumoniae* pneumonia (SMPP), it can be accompanied by a variety of intrapulmonary complications (e.g. pleural effusion, lung abscess, atelectasis, necrotizing pneumonia, and bronchiolitis obliterans) and extrapulmonary complications (e.g. myocarditis, nephritis, encephalitis, and hemolytic anemia), all of which have a profound impact on the daily life and wellbeing of infected children [3–5].

Pleural effusion, a well-recognized complication of MP infection [6], occurs in approximately 20.3% to 20.7% of *Mycoplasma pneumoniae* pneumonia (MPP) cases across all age groups [7, 8]. Previous studies demonstrated that MPP patients with pleural effusion were more severe than those without pleural effusion, tending to have higher CRP levels and longer fever duration [4, 9], indicating the pivotal role of pleural effusion in MPP. Although the underlying pathophysiology of pleural effusion in MPP has not yet been fully understood, it is postulated that its development in patients with MPP might be associated with direct invasion, continuum of the MP infection, or exaggerated immune responses [10]. However, studies regrading pleural effusion in MPP are limited. A previous study speculated that pleural effusion caused by MP infection can be categorized into two patterns: one characterized by the absence of the MP genome and lower concentrations of cytokines (e.g. interleukin (IL)-18 and IL-8), while the other characterized by persistent chest disease with the presence of the MP genome and higher levels of IL-18 and IL-8 [11]. However, these studies included small sample sizes. Additionally, studies comprehensively investigating the clinical characteristics difference between the absence and the presence of MP in pleural effusion in MPP are lacking. The availability of such information would be helpful in identifying the clinical significance of MP positive pleural effusion in MPP children. Hence, the purpose of this study was to elucidating the clinical relevance of MP status in pleural fluid in MPP children by comparing the clinical manifestations, laboratory findings, radiological characteristics and treatment modalities.

Materials and methods

Study population

This retrospective, single-center, observational study enrolled children with pleural effusion (PE) caused by MPP, who were admitted to Children's hospital, Zhejiang University School of Medicine between January 2015 and December 2019 and required a diagnostic/therapeutic thoracentesis. The present study was approved by the Ethics Committee of Children's Hospital, Zhejiang University School of Medicine (No. 2021-IRB-270), and written informed consent was obtained from parents or legal guardians of each patient.

Case definitions

The diagnostic criteria for pneumonia were the presence of at least one of respiratory symptoms and signs (e.g. fever, cough, productive sputum, dyspnea, chest pain, or abnormal lung auscultation) and evidence of a new pulmonary infiltrate on radiologic images (e.g. chest radiographs or CT scans). MP infection was diagnosed by positive results for MP polymerase chain reaction (PCR) tests of nasopharyngeal aspirates or bronchoalveolar lavage fluid (BALF) or pleural effusion. Pleural effusion was confirmed by imaging examinations (e.g. chest radiographs, CT scans or ultrasonographs).

Inclusion criteria were as follows: (1) met the diagnostic criteria; (2) pleural effusions were available for at least a diagnostic thoracentesis, or drained by thoracentesis with or without chest tube insertion for etiology analysis and symptom relief; (3) exclusion of other respiratory tract infections and tuberculosis.

We excluded patients with immunodeficiency disease, neurological disease, neuromuscular disease, congenital malformation, congenital heart disease, vascular ring malformation, bronchopulmonary dysplasia, primary ciliary dystrophy, bronchiolitis obliterans, pulmonary tumor, noninfectious interstitial pulmonary disease, cystic fibrosis, neoplasia, pulmonary tuberculosis, nosocomial pneumonia, other respiratory pathogen infections, or asthma as well as those whose pleural effusion caused by diseases other than CAP or data were incomplete.

To exclude the possibility of coinfection, additional tests were performed, including protein purified derivative (PPD), blood/pleural effusion/nasopharyngeal aspirate/ BALF cultures, nasopharyngeal aspirate for virus

antigen detection (respiratory syncytial viruses, influenza viruses, adenovirus, and parainfluenza virus), and serology for Chlamydia pneumoniae, Legionella pneumoniae, and Chlamydia trachomatis.

MPP patients with pleural effusions were divided into 2 groups depending on the presence or absence of MP DNA in pleural effusions. We retrospectively compared demographics (e.g. gender, age), clinical characteristics (e.g. fever, cough, chest pain, preadmission fever duration, total fever duration, and hospitalization days), laboratory findings (e.g. routine blood examination, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), D-dimer, cytokines, lactate dehydrogenase (LDH), procalcitonin (PCT), subpopulations of T lymphocytes, and immunoglobulins), and radiographic findings between the two groups. Patients received flexible bronchoscopy with bronchoalveolar lavage (BAL) according to the guide to pediatric bronchoscopy [12].

Measurement of serum cytokines

Venous blood samples for Th1/Th2 cytokines (including interleukin (IL)-2, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)- α and interferon (IFN)- γ) detection were collected from each subject at admission and tested by FACSCalibur flow cytometer (BD biosciences, San Diego, CA, USA) using a CBA Human Th1/Th2 Cytokine Kit II (BD biosciences, San Diego, CA, USA).

Pleural effusion detection and pleural fluid analysis

A diagnostic lung ultrasound was performed for identifying pleural effusions. This procedure required patients to maintain a comfortable, standard seated posture and try to keep a calm breath. The ultrasound probe was positioned perpendicularly to the skin for measuring the maximum interpleural distance (the distance between the visceral and mural pleura) and determining the optimal puncture site for further intervention. Patients with an interpleural distance > 10 mm were recommended to undergo thoracentesis. Conversely, for those with a small amount of pleural fluid (an interpleural distance ≤ 10 mm), thoracentesis was not advised due to the potential risks (e.g. pneumothorax or bleeding). Subsequently, thoracentesis was performed at the predetermined optimal puncture site without direct ultrasound guidance for draining excess pleural fluid. Pleural fluid samples were then subjected to routine analysis including total leukocyte count, differential white cell count, glucose, protein, LDH, adenosine deaminase (ADA), Gram stain, MP DNA detection and common bacterial culture.

Bronchoalveolar lavage under bronchoscopy

As mucus accumulation in the airways and bronchial cast formation have been frequently observed in MPP patients [13], flexible bronchoscopy with bronchoalveolar lavage (BAL) is of great assistance in the therapy for severe MPP and refractory MPP [14]. Flexible bronchoscopy with BAL were performed on MPP patients in the following situations: (i) children with radiologically proven large pulmonary lesions, (ii) children with recurrent/persistent atelectasis, (iii) children with suspected mucus plug or plastic bronchitis, and (iv) unresponsiveness to standard anti-MP therapy.

Parental consent was obtained and preoperative preparations were completed prior to the bronchoscopy procedure. Three types of flexible bronchoscopes were utilized, tailored to the age and body weight of the patients: Olympus (Japan) BFXP40 (2.8 mm external diameter and 1.2 mm working channel), BF-3C30 (3.6 mm, 1.2 mm), and BF-P40(4.9 mm, 2.2 mm). Flexible bronchoscopy with BAL were performed by experienced respiratory endoscopists as we previously described [14]. In brief, after fasting for > 6 h, patients were sedated through intravenous administration of midazolam at a dosage of 0.1–0.15 mg/kg, and further anesthesia was applied topically with 1% lidocaine to the nasal cavity, vocal cords, and trachea for optimal comfort and relaxation. The bronchoscope was wedged in the subsegmental bronchus of the affected lobe as identified on the chest radiograph. BAL with normal saline (weight < 20 kg: 1 mL/kg/time, 3 times; weight > 20 kg: 20 mL/time, 3 times) was conducted with -25 to -100 mmHg suction, in accordance with the Official American Thoracic Society Technical Standards [15]. Meanwhile, BALF was collected for cytological analysis and microbiological determinations, including MP DNA detection and cultures of bacteria, mycobacteria and fungi [16]. Throughout the procedure, vital signs including breathing frequency, heart rate, and pulse oxygen saturation (SpO2) were vigilantly monitored in real-time. In the event of hypoxia (cyanosis, low SpO2, and/or high heart rate), oxygen of appropriate concentration was given immediately, and the procedure was stopped temporarily when necessary.

Other treatments

On admission, patients received antibiotics, nebulized budesonide suspension combined with ipratropium bromide inhalation, and postural drainage through back patting. Methylprednisolone (2 mg/kg/day) or intravenous immunoglobulins (IVIG) (400 mg/kg/day) were administrated in critically ill patients as appropriate based on their individual clinical status.

Low molecular weight heparin (LMWH) was administered in MPP patients with a marked elevation in D-dimer levels. Specifically, the normal range for D-dimer is < 0.55 mg/L, and LMHW is recommended when these levels surge over tenfold, reaching or exceeding 5.5 mg/L in MPP patients. In MPP patients without pulmonary embolism, the recommended dose of LMWH is 100 U/kg administered once daily via subcutaneous injection for preventive anticoagulant therapy. Conversely, in MPP patients with pulmonary embolism, the dose of LMWH remains 100 U/kg but is administered every 12 h through subcutaneous injection for anticoagulant therapy.

Statistical analysis

Statistical analysis was performed using SPSS software, version 23.0 (SPSS Inc., Chicago, IL, USA). The normal distribution data were expressed as mean ± standard deviation and compared using Student's t-test, while the skewed distribution data were presented as medians and interquartile ranges (25th–75th percentiles) and compared using the Mann–Whitney U-test. Meanwhile, categorical variables were reported as percentages and compared using the Chi-square test or Fisher's exact test. Receiver operating characteristic (ROC) curves were created to evaluate candidate markers related to MPP with MP-DNA positive pleural effusion. Meanwhile, the areas under the curve (AUC) and predictive metrics, including sensitivity, specificity and optimal threshold values were calculated to ascertain the diagnostic accuracy and efficacy of these markers. A logistic regression analysis was conducted to identify risk factors for MP-DNA positive pleural effusion, concurrently estimating the odds ratio (OR) for each identified factor. Spearman's correlation coefficient was used to analyze the correlation between the parameters. A two-sided p-value of < 0.05 was considered statistically significant.

Results

Clinical characteristics of MPP patients with pleural effusion

A total of 165 MPP patients (78 males and 87 females) with pleural effusion fulfilled the inclusion criteria between January 2015 and December 2019. MP DNA was undetected in the pleural effusion of 127 cases (categorized as negative group), while the remaining 38 cases exhibited positive results of MP DNA detection in the pleural effusion (categorized as positive group).

We compared clinical characteristics of the two groups and the data are presented in Table 1. The proportion of female in the positive group was significantly higher than that in the negative group (71.05% vs. 47.24%, $p = 0.010$). The positive group showed significantly longer hospital stay (15.00 (10.75–19.25) vs. 11.00 (9.00–14.00), $p = 0.001$) and total course of illness (23.00 (18.00–28.00) vs. 20.00 (17.00–24.00), $p = 0.010$) compared to the negative group. There was no significant intergroup difference in the age, fever duration before admission, fever duration after admission, total fever duration, and course of disease before admission. All patients presented with symptoms of fever and cough. No significant differences were observed in the incidences of chest pain, abdominal pain, headache, tachypnea, and rash between the two groups. Pericardial effusion (23.7% vs. 7.9%, $p = 0.017$), atelectasis (73.7% vs. 53.5%, $p = 0.027$) and necrotizing pneumonia (23.7% vs. 7.9%, $p = 0.017$) occurred more frequently in the positive group than in the negative group. The occurrence of plastic bronchitis, peritoneal effusion, pulmonary embolism and splenic infarction showed no difference between the two groups. No cases complicated by pericardial effusion or peritoneal effusion required additional procedures for its removal.

Comparison of laboratory findings at the time of admission

As shown in Table 2, the levels of neutrophils percentages (82.35% (75.40%-85.78%) vs. 72.70%(64.30%-79.90%), $p < 0.001$), C-reactive protein (CRP) (71.12 (37.75–139.41) vs. 31.15 (13.54–65.00) mg/L, $p < 0.001$), procalcitonin (PCT) (0.65 (0.30–3.05) vs. 0.33 (0.17–1.13) ng/ml, $p = 0.005$), serum lactate dehydrogenase (LDH) (799.00 (589.00–1081.50) vs. 673.00 (503.00–869.00) U/L, $p = 0.009$), D-dimer (6.21 (3.37–16.11) vs. 3.32 (2.12–6.62) mg/L, $p = 0.001$) and CD19 + % (23.04 ± 9.04 vs. 19.65 ± 7.83, $p = 0.048$) on admission were significantly higher, and the level of lymphocytes% (12.35%(8.00%-15.72%) vs. 18.45%(12.93%-24.60%), $p < 0.001$), serum total protein (TP) (59.55 (55.93–64.30) vs. 62.25 (57.38–67.13) g/L, $p = 0.037$), CD3 + % (59.60% ± 11.09% vs. 68.14% ± 10.66%, $p < 0.001$), CD4 + % (29.22% ± 8.29% vs. 34.55% ± 8.24%, $p < 0.001$) and immunoglobulin G (IgG) (7.52 (5.81–8.80) vs. 8.85 (7.13–10.55) g/L, $p = 0.003$) were significantly lower in the positive group than in the negative group. The rest examined variants (e.g. IL-2, IL-4 and IL-6) in the positive group showed no differences from those of the negative group. Additionally, no notable variations were observed in the proportions of lymphocytes, neutrophils, macrophages and eosinophils in bronchoalveolar lavage fluid (BALF) between the two groups.

Pleural fluid analysis

All enrolled patients underwent thoracocentesis followed by pleural fluid routine examination, however, none of them required closed thoracic drainage. The pleural fluid analysis data are presented in Table 3. The positive group underwent a notably higher frequencies of thoracentesis

Table 1 Comparison of clinical characteristics according to the presence or absence of MP DNA in pleural effusion in children with MPP

Variables	negative ($n=127$)	positive ($n=38$)	p
Gender(male/female)	67/60	11/27	0.010
Age (Year)	7.00 (5.33–8.67)	6.02 (5.19–8.63)	0.257
Hospital stay (day)	11.00 (9.00–14.00)	15.00 (10.75–19.25)	0.001
Fever duration before admission (day)	8.00 (6.00–10.00)	7.00 (6.00–10.00)	0.668
Fever duration after admission (day)	4.00 (2.00–6.00)	5.00 (3.00–7.00)	0.155
Total fever duration (day)	12.00 (10.00–14.00)	13.00 (10.00–15.00)	0.360
course of illness before admission (day)	9.00 (7.00–10.00)	8.00 (6.75–10.00)	0.530
Total course of illness (day)	20.00 (17.00–24.00)	23.00 (18.00–28.00)	0.010
Presenting manifestation			
fever	127 (100.0%)	38 (100.0%)	NA
cough	127 (100.0%)	38 (100.0%)	NA
chest pain	1 (0.8%)	0 (0.0%)	1.000
abdominal pain	2 (1.6%)	0 (0.0%)	1.000
headache	2 (1.6%)	0 (0.0%)	1.000
tachypnea	36 (28.4%)	13 (34.2%)	0.488
rash	6 (4.7%)	2 (5.3%)	1.000
Other complications			
Pericardial effusion	10 (7.9%)	9 (23.7%)	0.017
Peritoneal effusion	13 (10.2%)	5 (13.2%)	0.566
Atelectasis	68 (53.5%)	28 (73.7%)	0.027
Necrotizing pneumonia	10 (7.9%)	9 (23.7%)	0.017
Pulmonary embolism	2 (1.6%)	1 (2.6%)	0.547
Splenic infarction	1 (0.8%)	0 (0.0%)	1.000
Plastic bronchitis	6 (4.7%)	4 (10.5%)	0.241

NA Not applicable

procedures in comparisons to the negative group (1.00 (1.00–2.00) vs. 1.00 (1.00–1.00), $p=0.049$). Additionally, marked elevations in total protein (TP) (40.94 ± 5.78 vs. 38.64 ± 6.04 g/L, $p=0.039$), adenosine deaminase (ADA) (49.25 (36.20–60.18) vs. 36.20 (28.10–46.50) U/L, $p<0.001$), and LDH (2298.50 (1259.75–3287.00) vs. 1199.00 (707.00–1761.00) U/L) levels were detected in pleural fluid of the positive group when compared to the negative group. However, no significant differences were observed between the two group in terms of the maximum amount of pleural effusion and its location. The regression period of pleural effusion (11.00 (7.00–23.00) vs. 9.00 (7.00–13.00) days, $p=0.034$) was significantly protracted in the positive group compared to the negative group.

To gain a deeper insight into the relationship between ADA level and disease course, Spearman's correlation analysis was carried out. Pleural fluid ADA level was positively correlated with length of hospitalization ($r=0.277$, $p<0.001$) and total disease course ($r=0.284$, $p<0.001$). Inversely, there was no significantly correlation between serum ADA level with length of hospitalization ($r=$-0.101, $p=0.198$) and total disease course ($r=$-0.072, $p=0.361$).

Treatment

All MPP patients were successfully treated regardless of the presence or absence of MP DNA in pleural effusion. As shown in Table 4, all patients received azithromycin treatment. One noticeable trend was that the number of patients on low molecular weight heparin (LMWH) therapy (9 (23.7%) vs. 12 (9.4%), $p=0.028$) was higher in the positive group. However, there were no significant differences between the two groups in terms of in the requirement for oxygen support, administration of systemic corticosteroids or immunoglobulin (IVIG), intervention with flexible bronchoscopy or the frequencies of bronchoscopies performed. Furthermore, the duration of systemic steroids administration in the positive group was longer than that in the negative group (4.50 (0.00–16.00) vs. 0.00 (0.00–9.00) days, $p=0.042$), whereas no statistical significance was observed in the length of azithromycin treatment or the period of oxygen inhalation between the two groups.

Table 2 Comparison of laboratory findings according to the presence or absence of MP DNA in pleural effusion in children with MPP

Variables	negative ($n=127$)	positive ($n=38$)	*p*
Blood routine test			
WBC ($\times 10^9$/L)	7.78 ± 3.49	8.78 ± 2.93	0.108
L (%)	18.45 (12.93–24.60)	12.35 (8.00–15.72)	< 0.001
N (%)	72.70 (64.30–79.90)	82.35 (75.40–85.78)	< 0.001
PLT ($\times 10^9$/L)	260.00 (202.75–330.50)	249.50 (173.00–294.00)	0.287
CRP (mg/L)	31.15 (13.54–65.00)	71.12 (37.75–139.41)	< 0.001
Blood biochemical test			
TP (g/L)	62.25 (57.38–67.13)	59.55 (55.93–64.30)	0.037
ALB (g/L)	33.30 ± 4.64	31.77 ± 4.62	0.077
Globulin (g/L)	28.80 (25.40–32.70)	28.50 (28.48–35.18)	0.152
A/G	1.16 ± 0.27	1.16 ± 0.26	1.000
ALT (U/L)	36.00 (21.00–78.00)	31.00 (19.75–89.75)	0.971
AST (U/L)	60.00 (42.00–108.00)	71.50 (45.00–122.00)	0.363
ADA (U/L)	25.40 (22.40–29.8)	24.25 (21.58–29.23)	0.420
LDH (U/L)	673.00 (503.00–869.00)	799.00 (589.00–1081.50)	0.009
CK-MB (U/L)	25.00 (17.00–36.00)	28.00 (20.00–43.25)	0.123
Cytokine			
IL-2 (pg/ml)	2.50 (1.50–3.70)	2.55 (1.78–3.90)	0.559
IL-4 (pg/ml)	2.50 (1.85–3.45)	2.25 (1.73–3.00)	0.475
IL-6 (pg/ml)	44.90 (16.75–85.55)	60.10 (24.48–197.18)	0.191
IL-10 (pg/ml)	9.20 (6.30–13.15)	10.40 (5.38–16.25)	0.623
TNF-α (pg/ml)	2.10 (1.55–2.80)()	2.05 (1.30–2.53)	0.381
IFN-γ (pg/ml)	15.20 (6.05–42.25)	21.50 (7.38–83.47)	0.129
Cellular immunity			
CD19 + (%)	19.65 ± 7.83	23.04 ± 9.04	0.048
CD3 + (%)	68.14 ± 10.66	59.60 ± 11.09	< 0.001
CD4 + (%)	34.55 ± 8.24	29.22 ± 8.29	< 0.001
CD8 + (%)	28.74 ± 6.52	26.50 ± 7.85	0.107
CD3-CD16 + CD56 + (%)	5.60 (3.30–8.45)	6.80 (3.40–11.05)	0.225
CD4 + /CD8 + (%)	1.14 (0.97–1.45)	1.17 (0.83–1.52)	0.672
Immunoglobulin			
IgG (g/L)	8.85 (7.13–10.55)	7.52 (5.81–8.80)	0.003
IgA (g/L)	1.37 (1.02–1.97)	1.36 (0.82–1.74)	0.204
IgM (g/L)	1.53 (1.01–2.37)	1.19 (0.93–1.76)	0.210
C3 (g/L)	1.20 ± 0.23	1.15 ± 0.24	0.358
C4 (g/L)	0.35 (0.22–0.47)	0.32 (0.22–0.42)	0.332
IgE (IU/ml)	101.50 (46.45–332.25)	132.00 (69.20–311.00)	0.611
MP-IgM (COI)	2.75 (1.04–6.12)	2.58 (1.02–4.86)	0.997
D-dimer (mg/L)	3.32 (2.12–6.62)	6.21 (3.37–16.11)	0.001
PCT (ng/ml)	0.33 (0.17–1.13)	0.65 (0.30–3.05)	0.005
ESR (mm/h)	32.00 (19.00–47.00)	28.50 (23.00–43.50)	0.922
Cytology of BALF			
L%	4.00 (3.00–7.00)	5.00 (3.00–10.00)	0.117
N%	20.00 (5.25–33.75)	20.00 (6.75–40.50)	0.488
M%	75.00 (59.63–86.00)	69.00 (50.00–87.50)	0.644
E%	0.00 (0.00–0.50)	0.00 (0.00–0.00)	0.280

WBC White blood cell, *L* Lymphocyte, *N* Neutrophil, *PLT* Platelet, *CRP* C-reactive protein, *TP* Total protein, *ALB* Albumin, *A/G* Albumin/globulin, *ALT* Alanine aminotransferase, *AST* Aspartate aminotransferase, *ADA* Adenosine deaminase, *LDH* Lactate dehydrogenase, *CK-MB* Creatine kinase-MB, *IL* Interleukin, *TNF* Tumor necrosis factor, *IFN* Interferon, *Ig* Immunoglobulin, *C* Complement, *PCT* Procalcitonin, *ESR* Erythrocyte sedimentation rate, *BALF* Bronchoalveolar lavage fluid, *M* Macrophage, *E* Eosinophil

Table 3 Comparison of pleural fluid findings according to the presence or absence of MP DNA in pleural effusion in children with MPP

Variables	negative ($n=127$)	positive ($n=38$)	p
Frequencies of thoracentesis (number)	1.00 (1.00–1.00)	1.00 (1.00–2.00)	0.049
Frequencies of thoracentesis (once / ≥ twice)	108/19	27/11	0.050
Maximum amount of pleural effusion (cm)	1.95 (1.60–2.80)	2.50 (1.45–3.35)	0.415
Location of pleural effusion(left/right/both)	35/27/65	6/8/24	0.300
Recovery Timeline for pleural effusion (days)	9.00 (7.00–13.00)	11.00 (7.00–23.00)	0.034
Pleural fluid			
WBC ($\times 10^6$/L)	753.00 (395.50–1504.50)	675.00 (400.00–1151.00)	0.424
PMN (%)	20.00 (9.00–35.00)	23.00 (10.00–39.25)	0.470
L (%)	80.00 (65.00–92.00)	77.00 (60.75–90.00)	0.413
TP (g/L)	38.64 ± 6.04	40.94 ± 5.78	0.039
ADA (U/L)	36.20 (28.10–46.50)	49.25 (36.20–60.18)	< 0.001
LDH (U/L)	1199.00 (707.00–1761.00)	2298.50 (1259.75–3287.00)	< 0.001
Glu (mmol/L)	6.86 (6.07–8.33)	6.93 (5.88–8.73)	0.810

WBC White blood cell, *PMN* Polymorphonuclear leukocyte, *L* Lymphocyte, *TP* Total protein, *ADA* Adenosine deaminase, *LDH* Lactate dehydrogenase, *Glu* Glucose

Table 4 Comparison of treatment according to the presence or absence of MP DNA in pleural effusion in children with MPP

Variables	negative ($n=127$)	positive ($n=38$)	p
Azithromycin	127 (100.0%)	38 (100.0%)	NA
Administration duration of azithromycin (days)	9.00 (7.25–10.00)	8.00 (7.00–10.00)	0.947
Systemic steroids	55 (43.3%)	22 (57.9%)	0.114
Administration duration of Systemic steroids (days)	0.00 (0.00–9.00)	4.50 (0.00–16.00)	0.042
Oxygen support	47 (37.0%)	17 (44.7%)	0.391
Duration of oxygen inhalation (days)	0.00 (0.00–4.00)	0.00 (0.00–7.25)	0.154
LMWH	12 (9.4%)	9 (23.7%)	0.028
IVIG	6 (4.7%)	5 (13.2%)	0.129
Flexible bronchoscopy	114 (89.8%)	34 (89.5%)	1.000
Frequencies of bronchoscopies (times)	1.00 (1.00–1.00)	1.00 (1.00–2.00)	0.566

NA Not applicable, *LMWH* Low molecular weight heparin, *IVIG* Immunoglobulin

Predictive values of the independent correlation factors in MPP patients with MP DNA positive in pleural effusion

The ROC analysis was employed to explore predictive values of laboratory data for MPP with MP DNA-positive pleural effusion, and the optimal cut-off value with maximum sensitivity and specificity was also determined in Fig. 1. ROC analysis revealed that neutrophil percentage, CRP, D-dimer, pleural fluid ADA and pleural fluid LDH were of great significance in the diagnosis of MPP with MP DNA-positive pleural effusion, with areas under the curve exceeding 0.7. When the cut-off value for the neutrophil percentage, CRP, D-dimer, pleural fluid ADA and pleural fluid LDH was set at 77.7%, 66.4 mg/L, 7.33 mg/L, 42.5 U/Land 1801.0 U/L, respectively, the diagnostic sensitivity and specificity for MPP with MP DNA-positive pleural effusion were as follows: 73.7% and 66.9% for neutrophil percentage, 60.5% and 77.2% for CRP, 46.7% and 86.2% for D-dimer, 71.1% and 70.1% for pleural fluid ADA, 65.8% and 76.4% for pleural fluid LDH, respectively (Table 5).

Logistic regression analysis for the related factors predicting the MPP with MP DNA-positive pleural effusion

To further evaluate the predictors associated with MPP with MP DNA-positive pleural effusion, a multiple logistic regression analysis was conducted. Notably, D-dimer > 7.33 mg/L emerged as a crucial predictor, exhibiting an odds ratio (OR) value of 3.517 (Table 6).

Discussion

The present study demonstrated a more severe clinical course in MPP patients with MP-positive pleural effusion when compared to those with MP-negative pleural effusion, reflected by a prolonged hospitalization stay,

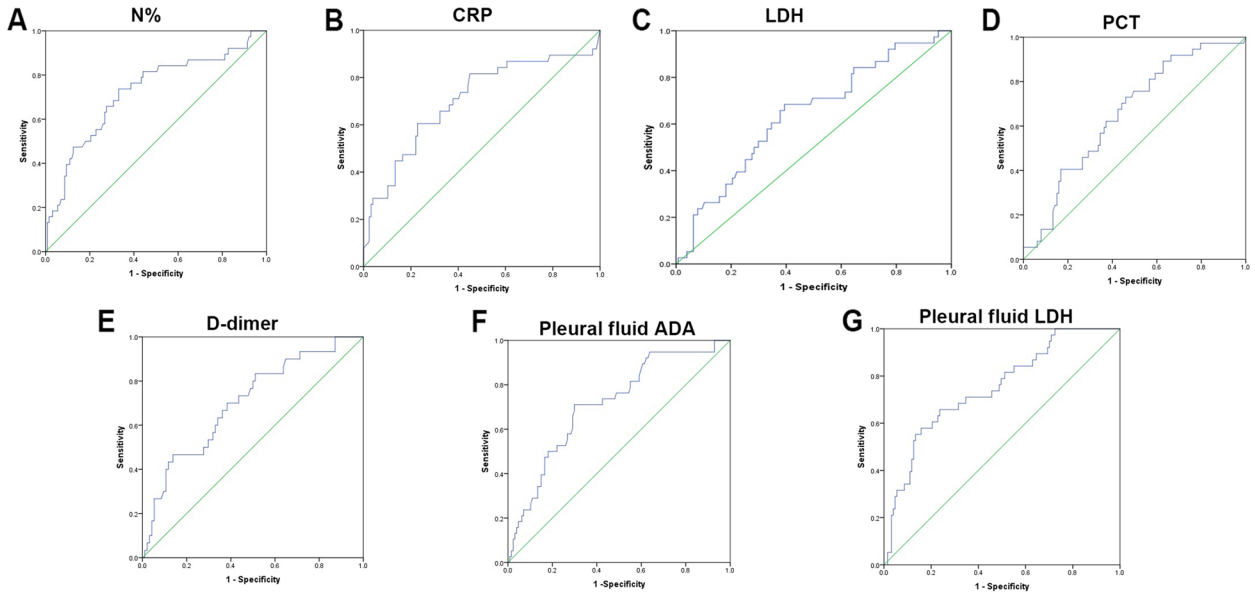

Fig. 1 Predictability of the independent factors for MPP with MP DNA positive in pleural effusion. ROC curve of the N% (**A**), CRP (**B**), LDH (**C**), PCT (**D**), D-dimer (**E**), Pleural fluid ADA (**F**) and Pleural fluid LDH (**G**)

Table 5 Predictive values of the independent correlation factors for MPP with MP DNA positive in pleural effusion

Independent factors	AUC	95%CI	*p*	Cut-off value	Sensitivity	Specificity
N (%)	0.722	0.624–0.820	< 0.001	77.7	73.7%	66.9%
CRP (mg/L)	0.704	0.600–0.808	< 0.001	66.4	60.5%	77.2%
Serum LDH (U/L)	0.640	0.540–0.740	0.009	713.5	68.4%	60.6%
PCT (ng/ml)	0.656	0.560–0.752	0.005	0.34	73.0%	54.0%
D-dimer (mg/L)	0.702	0.595–0.808	0.001	7.33	46.7%	86.2%
Pleural fluid ADA (U/L)	0.713	0.622–0.805	< 0.001	42.5	71.1%	70.1%
Pleural fluid LDH (U/L)	0.748	0.661–0.836	< 0.001	1801.0	65.8%	76.4%

N Neutrophil, *CRP* C-reactive protein, *LDH* Lactate dehydrogenase, *PCT* Procalcitonin, *ADA* Adenosine deaminase, *AUC* Areas under the curve, *CI* Confidence interval

Table 6 Stepwise logistic regression analysis for the related factors predicting the MPP with MP DNA positive in pleural effusion

Variable	B	S.E	Wald	*p*	OR	95%CI
N (%)	0.501	0.576	0.755	0.385	1.650	0.533–5.106
CRP (mg/L)	0.582	0.551	1.119	0.290	1.790	0.608–5.269
Serum LDH (U/L)	0.634	0.529	1.437	0.231	1.885	0.669–5.316
PCT (ng/ml)	0.130	0.533	0.060	0.807	1.139	0.401–3.241
D-dimer (mg/L)	1.258	0.604	4.339	0.037	3.517	1.077–11.486
Pleural fluid ADA (U/L)	0.848	0.611	1.925	0.165	2.334	0.705–7.729
Pleural fluid LDH (U/L)	-0.090	0.685	0.017	0.895	0.913	0.238–3.499

N Neutrophil, *CRP* C-reactive protein, *LDH* Lactate dehydrogenase, *PCT* Procalcitonin, *ADA* Adenosine deaminase, *OR* Odds ratio, *CI* Confidence interval

an extend total disease duration, increased incidence of other complications (e.g. pericardial effusion, atelectasis, necrotizing pneumonia), significant abnormalities in laboratory indicators (e.g. N%, CRP, PCT, LDH, D-dimer), longer duration of systemic steroids administration and a higher requirement for LMWH therapy. Notably, N%, CRP, PCT, LDH and D-dimer are indicators of inflammation, and the occurrence of pericardial effusion, atelectasis and necrotizing pneumonia was associated with the systemic inflammatory response to MP infection [17]. Currently, the widely accepted theory is that excessive immune response is responsible for MPP progression [18–20]. Similarly, Narita et al. supposed that the detection of MP in the pleural effusion was strongly

associated with delay resolution of chest radiographic abnormality in MPP children [10]. Taken together, these evidences suggested a stronger systemic immune-inflammatory reaction in MPP patients with MP-positive pleural effusion, which leads further to a longer hospital-stays and illness duration.

Unlike bacterial pleural effusion with polymorphonuclear leukocyte (PMN) predominance, pleural effusion in MPP patients was mostly lymphocyte-predominant with a high ADA level [21]. Consistently, regardless of the presence or absence of MP in pleural effusion, pleural fluid analysis revealed lymphocyte-predominant exudates in MPP patients in our study. This could partially be explained by the pathological finding that MP is characterized by lymphoplasmacytic infiltrates in the bronchiolar wall, along with peribronchial wall thickening [22]. The persistent inflammation of underlying lung parenchyma induced by mononuclear cells might cause lymphocyte-predominant pleural effusion. It is noteworthy that we showed MPP patients with MP-positive pleural effusion exhibited higher ADA levels compared to those with MP-negative pleural effusion. ADA has 2 major isoenzymes, namely ADA1 and ADA2. ADA1 is ubiquitous in all cells, including lymphocytes and monocytes [23], and is mainly responsible for total ADA elevation in complex, purulent or some malignant pleural effusions [24]. Conversely, ADA2 is exclusively expressed in monocytes and macrophages [23], and upregulated when these cells are infected by intracellular microorganisms, such as *Mycobacterium tuberculosis* [25, 26]. We speculate that high ADA activity in the pleural effusion of MPP originates from monocytes and macrophages, and the presence of MP in pleural effusion further augments the activity of these cells. We showed the length of hospitalization and total disease course were positively correlated with pleural fluid ADA, suggesting higher pleural fluid ADA corresponded to longer the hospitalization duration and total disease course. Moreover, for predicting MPP with MP-DNA positive pleural effusion, the AUC for pleural fluid ADA was 0.713, and the optimal cutoff point was 42.5 U/L, with a sensitivity of 71.1% and specificity of 70.1% in our research, implying pleural fluid ADA is a critical biomarker for measuring MP infection status. Notably, as ADA levels also significantly elevated in tuberculosis, an increase in pleural fluid ADA level can further complicate the differential diagnosis between tuberculosis and MPP [25, 27]. In other words, ADA in pleural effusion is not a specific marker for MPP. Therefore, the predictive value of ADA depends not only on its sensitivity and specificity, but also on the local prevalence of pathogens. Further studies are necessary to distinguish MP pleural effusion from tuberculosis pleural effusion.

Persistent and excessive inflammatory reaction could cause tissue damage and cell death, which results in release of LDH [28]. Our study revealed a notable increase in LDH levels in both serum and pleural effusion in MPP patients with MP-positive pleural effusion, indicating both intrapulmonary and extrapulmonary damage due to stronger excessive local and systematic host-cellular responses to MP infection compared to MP-negative pleural effusion cases. Furthermore, a previous study reported that LDH increased in parallel with the severity of MPP and was documented as a valuable indicator for evaluating MPP conditions [29]. Consistently, we showed both serum and pleural fluid LDH had predictive value for MP-positive pleural effusion. Altogether, these data indicated that LDH play a pivotal role in assessing the severity of MP infection.

D-dimer, a specific marker of the fibrinolytic system, has been found increased significantly after MP infection and is more pronounced in SMPP or RMPP [30]. Likewise, D-dimer was significantly higher in the MP-positive pleural effusion group than in the MP-negative pleural effusion, demonstrating a more hypercoagulable state in the positive group. Although the precise mechanism underlying abnormal coagulation function in MP infection remains elusive, it is plausible that MP triggers an extensive synthesis and secretion of cytokines (e.g. interleukins, tumor necrosis factors and chemokines), then disrupting the delicate imbalance between the blood coagulation and anticoagulation systems, ultimately leading to local vascular damage and subsequent accumulation of metabolites (e.g. D-dimer) [31]. In accordance with the previous study [11], MP genome detectable in pleural effusion in our study exacerbated the local and systemic inflammation and aggravated disorders of the coagulation system. Furthermore, Li et al. revealed that the degree of elevated D-dimer was positively correlated with the severity of MPP, and elevated serum D-dimer levels (> 3.705 mg/L) serving as an independent predictor of MPP combined with necrotizing pneumonia [32]. Similarly, our study showed that a D-dimer level of > 7.33 mg/L was a risk factor for the development of MP-positive pleural effusion in MPP patients, suggesting a hypercoagulable state not only contributed to microthrombus in pulmonary circulation but also was closely associated with the inflammatory response and the severity of MP infection [33]. Therefore, D-dimer levels could assist clinicians in precisely evaluating the disease status, promptly identifying serious pulmonary complications and initiating early comprehensive treatment measures to shorten the disease duration and improve the prognosis.

In line with previous reports [9, 29, 30, 34], we evaluated several easy-to-measure serum inflammatory markers

(e.g. neutrophils, LDH, PCT, CRP, D-dimer) to determine their capability in distinguishing between the positive group and the negative group. These evidences highlighted the pivotal role these markers play in providing a better understanding of the clinical course and risk stratification of patients with MPP. It has been suggested that MP-related acute lung injury is not only attributed to the presence of MP itself but also results from an excessive host immune reaction [35]. We revealed that the pleural fluid MP DNA positive group experienced a longer duration of glucocorticoid therapy, implying hyperimmune inflammatory responses and suggesting the dose of methylprednisolone at 2 mg/kg/d may be insufficient for these patients. Dosage escalation of methylprednisolone should be considered for MPP patients with MP-positive pleural effusion to optimize inflammatory control and expedite recovery. Additionally, Guo et al. demonstrated serum LDH and ferritin levels as useful biomarkers for determining the appropriate corticosteroid dosage in treating children with RMPP [36]. Likewise, Xu et al. suggested the optimal values of CRP, LDH, and neutrophils (CRP 44.45 mg/L, LDH 590 IU/L, neutrophils 73.75%) may be the valuable predictors of using methylprednisolone pulse therapy. Collectively, it is worthy of refining the indication, timing and dosage of glucocorticoid administration for different MPP subtypes for reducing the intensity of local inflammation, alleviating the immune reaction and promoting disease recovery with the guide of comprehensive analysis of inflammatory parameters in the future clinical practice.

The advantage of this study is that all five markers studied, namely neutrophil percentage, CRP, LDH, PCT and D-dimer, can be easily detected, quantified, and calculated, enabling a rapid evaluation of severity and prognosis of MPP when combined with clinical manifestations. Still, our study had several limitations. Firstly, it exclusively included MPP patients with pleural effusion who underwent thoracentesis, potentially reflecting a selection bias towards more severe cases within the study population. However, pleural effusion frequently occurs in more severe MPP cases; thus, the findings of our study are applicable to real-world clinical scenarios. Secondly, since our study was single-center and retrospective, a multicenter, prospective cohort study is crucial to validating our findings. Such a study would help improve the prognosis of pediatric MPP in the era of growing macrolide resistance of MP and RMPP. Thirdly, MP was not screened for macrolide-resistant. Lastly, since the immune status of asthma patients may have a different pattern in the course of MPP [37, 38], our study excluded asthma patients. Further research will be needed to explore the characteristics of MPP with pleural effusion in asthmatic children.

Conclusions

The present study highlighted differences in clinical features between MPP subtypes with and without MP DNA in pleural effusion, of which MP-DNA positive effusion linked to a more severe clinical course. D-dimer > 7.33 mg/L was a high risk factor for MP-positive effusion in MPP. The results of this study may provide valuable guidance for the early management of MP infection and prediction of the clinical course of MPP in children.

Abbreviations

ALB	Albumin
A/G	Albumin/globulin
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ADA	Adenosine deaminase;
BALF	Bronchoalveolar lavage fluid
C	Complement
CI	Confidence interval
CRP	C-reactive protein
CK-MB	Creatine kinase-MB
E	Eosinophil
ESR	Erythrocyte sedimentation rate
IL	Interleukin
IFN	Interferon
Ig	Immunoglobulin
IVIG	Immunoglobulin
L	Lymphocyte
LDH	Lactate dehydrogenase
LMWH	Low molecular weight heparin
M	Macrophage
N	Neutrophil
NA	Not applicable
OR	Odds ratio
PLT	Platelet
PCT	Procalcitonin
PMN	Polymorphonuclear leukocyte
TP	Total protein
WBC	White blood cell
TNF	Tumor necrosis factor

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Authors' contributions

SL and JZ analyzed the data and prepared the manuscript. JH, DY and GZ performed the data collection and analysis. LT and ZC conceived and designed the study and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate

This study involving human participants were reviewed and approved by Ethics Committee of Children's Hospital, Zhejiang University School of Medicine (No. 2021-IRB-270). Parents or legal guardians of each patient provided their written informed consent to participate in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Liu WK, Liu Q, Chen DH, Liang HX, Chen XK, Chen MX, Qiu SY, Yang ZY, Zhou R. Epidemiology of acute respiratory infections in children in Guangzhou: a three-year study. PLoS ONE. 2014;9:e96674.
2. Jain S, Williams DJ, Arnold SR, Ampofo K, Bramley AM, Reed C, Stockmann C, Anderson EJ, Grijalva CG, Self WH, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. N Engl J Med. 2015;372:835–45.
3. Gao LW, Yin J, Hu YH, Liu XY, Feng XL, He JX, Liu J, Guo Y, Xu BP, Shen KL. The epidemiology of paediatric Mycoplasma pneumoniae pneumonia in North China: 2006 to 2016. Epidemiol Infect. 2019;147:e192.
4. Lee KL, Lee CM, Yang TL, Yen TY, Chang LY, Chen JM, Lee PI, Huang LM, Lu CY. Severe Mycoplasma pneumoniae pneumonia requiring intensive care in children, 2010–2019. J Formos Med Assoc. 2021;120:281–91.
5. Moynihan KM, Barlow A, Nourse C, Heney C, Schlebusch S, Schlapbach LJ. Severe mycoplasma pneumoniae infection in children admitted to pediatric intensive care. Pediatr Infect Dis J. 2018;37:e336–8.
6. Nagayama Y, Sakurai N, Yamamoto K. Clinical observations of children with pleuropneumonia due to Mycoplasma pneumoniae. Pediatr Pulmonol. 1990;8:182–7.
7. Fine NL, Smith LR, Sheedy PF. Frequency of pleural effusions in mycoplasma and viral pneumonias. N Engl J Med. 1970;283:790–3.
8. Miyashita N, Sugiu T, Kawai Y, Oda K, Yamaguchi T, Ouchi K, Kobashi Y, Oka M. Radiographic features of Mycoplasma pneumoniae pneumonia: differential diagnosis and performance timing. BMC Med Imaging. 2009;9:7.
9. Luo XQ, Luo J, Wang CJ, Luo ZX, Tian DY, Xie XH. Clinical features of severe Mycoplasma pneumoniae pneumonia with pulmonary complications in childhood: a retrospective study. Pediatr Pulmonol. 2023;58:2815–22.
10. Narita M, Matsuzono Y, Itakura O, Yamada S, Togashi T. Analysis of mycoplasmal pleural effusion by the polymerase chain reaction. Arch Dis Child. 1998;78:67–9.
11. Narita M, Tanaka H. Two distinct patterns of pleural effusions caused by Mycoplasma pneumoniae infection. Pediatr Infect Dis J. 2004;23:1069 ; author reply 1069.
12. Sachdev A, Chhawchharia R. Flexible fiberoptic bronchoscopy in pediatric practice. Indian Pediatr. 2019;56:587–93.
13. Huang L, Huang X, Jiang W, Zhang R, Yan Y, Huang L. Independent predictors for longer radiographic resolution in patients with refractory Mycoplasma pneumoniae pneumonia: a prospective cohort study. BMJ Open. 2018;8:e023719.
14. Zhang Y, Chen Y, Chen Z, Zhou Y, Sheng Y, Xu D, Wang Y. Effects of bronchoalveolar lavage on refractory Mycoplasma pneumoniae pneumonia. Respir Care. 2014;59:1433–9.
15. Faro A, Wood RE, Schechter MS, Leong AB, Wittkugel E, Abode K, Chmiel JF, Daines C, Davis S, Eber E, et al. Official American thoracic society technical standards: flexible airway endoscopy in children. Am J Respir Crit Care Med. 2015;191:1066–80.
16. Murdoch DR, O'Brien KL, Driscoll AJ, Karron RA, Bhat N, Pneumonia Methods Working G, Team PC. Laboratory methods for determining pneumonia etiology in children. Clin Infect Dis. 2012;54(Suppl 2):S146-152.
17. Waites KB, Xiao L, Liu Y, Balish MF, Atkinson TP. Mycoplasma pneumoniae from the respiratory tract and beyond. Clin Microbiol Rev. 2017;30:747–809.
18. Yan C, Xue G, Zhao H, Feng Y, Li S, Cui J, Ni S, Sun H. Molecular and clinical characteristics of severe Mycoplasma pneumoniae pneumonia in children. Pediatr Pulmonol. 2019;54:1012–21.
19. Zhang Y, Zhou Y, Li S, Yang D, Wu X, Chen Z. The clinical characteristics and predictors of refractory mycoplasma pneumoniae pneumonia in children. PLoS ONE. 2016;11:e0156465.
20. Sekine H, Taguchi H, Watanabe H, Kawai S, Fujioka Y, Goto H, Kobayashi H, Kamiya S. Immunological analysis and pathological examination of gnotobiotic mice monoassociated with Mycoplasma pneumoniae. J Med Microbiol. 2009;58:697–705.
21. Cha SI, Shin KM, Jeon KN, Yoo SS, Lee J, Lee SY, Kim CH, Park JY, Jung TH. Clinical relevance and characteristics of pleural effusion in patients with Mycoplasma pneumoniae pneumonia. Scand J Infect Dis. 2012;44:793–7.
22. Rollins S, Colby T, Clayton F. Open lung biopsy in Mycoplasma pneumoniae pneumonia. Arch Pathol Lab Med. 1986;110:34–41.
23. Perez-Rodriguez E, Jimenez CD. The use of adenosine deaminase and adenosine deaminase isoenzymes in the diagnosis of tuberculous pleuritis. Curr Opin Pulm Med. 2000;6:259–66.
24. Krenke R, Korczynski P. Use of pleural fluid levels of adenosine deaminase and interferon gamma in the diagnosis of tuberculous pleuritis. Curr Opin Pulm Med. 2010;16:367–75.
25. Greco S, Girardi E, Masciangelo R, Capoccetta GB, Saltini C. Adenosine deaminase and interferon gamma measurements for the diagnosis of tuberculous pleurisy: a meta-analysis. Int J Tuberc Lung Dis. 2003;7:777–86.
26. Gakis C. Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2: diagnostic and biological role. Eur Respir J. 1996;9:632–3.
27. Trajman A, Pai M, Dheda K, van Zyl SR, Zwerling AA, Joshi R, Kalantri S, Daley P, Menzies D. Novel tests for diagnosing tuberculous pleural effusion: what works and what does not? Eur Respir J. 2008;31:1098–106.
28. Zhou Y, Qi M, Yang M. Current status and future perspectives of lactate dehydrogenase detection and medical implications: a review. Biosensors (Basel). 2022;12:12.
29. Kawamata R, Yokoyama K, Sato M, Goto M, Nozaki Y, Takagi T, Kumagai H, Yamagata T. Utility of serum ferritin and lactate dehydrogenase as surrogate markers for steroid therapy for Mycoplasma pneumoniae pneumonia. J Infect Chemother. 2015;21:783–9.
30. Shen F, Dong C, Zhang T, Yu C, Jiang K, Xu Y, Ning J. Development of a nomogram for predicting refractory mycoplasma pneumoniae pneumonia in children. Front Pediatr. 2022;10:813614.
31. Sarathchandran P, Al Madani A, Alboudi AM, Inshasi J. Mycoplasma pneumoniae infection presenting as stroke and meningoencephalitis with aortic and subclavian aneurysms without pulmonary involvement. BMJ Case Rep. 2018;2018:bcr2017221831.
32. Li YT, Zhang J, Wang MZ, Ma YM, Zhi K, Dai FL, Li SJ. Changes in coagulation markers in children with Mycoplasma pneumoniae pneumonia and their predictive value for Mycoplasma severity. Ital J Pediatr. 2023;49:143.
33. Li T, Yu H, Hou W, Li Z, Han C, Wang L. Evaluation of variation in coagulation among children with Mycoplasma pneumoniae pneumonia: a case-control study. J Int Med Res. 2017;45:2110–8.
34. Zhang Z, Wan R, Yuan Q, Dou H, Tu P, Shi D, Fu X, Xin D. Cell damage and neutrophils promote the infection of Mycoplasma pneumoniae and inflammatory response. Microb Pathog. 2022;169:105647.
35. Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. Clin Microbiol Rev. 2004;17:697–728 table of contents.
36. Wei D, Zhao Y, Zhang T, Xu Y, Guo W. The role of LDH and ferritin levels as biomarkers for corticosteroid dosage in children with refractory Mycoplasma pneumoniae pneumonia. Respir Res. 2024;25:266.
37. Chung HL, Shin JY, Ju M, Kim WT, Kim SG. Decreased interleukin-18 response in asthmatic children with severe Mycoplasma pneumoniae pneumonia. Cytokine. 2011;54:218–21.
38. Shin JE, Cheon BR, Shim JW, Kim DS, Jung HL, Park MS, Shim JY. Increased risk of refractory Mycoplasma pneumoniae pneumonia in children with atopic sensitization and asthma. Korean J Pediatr. 2014;57:271–7.

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