BMC Pulmonary Medicine

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Significance of respiratory virus coinfection in children with Mycoplasma pneumoniae pneumonia

Aosong Yu[1], Lingyi Ran[2], Xiaojia Sun[2] and Tong Feng[1*]

Abstract

Objective Mycoplasma pneumoniae is a major causative pathogen in community-acquired pneumonia. Respiratory viral coinfections in children with Mycoplasma pneumoniae pneumonia (MPP) are not uncommon and cause severe clinical manifestations. This study aims to investigate the impacts of viral coinfection in MPP patients and hopes to offer novel insights for discriminating between MPP and MPP coinfection.

Methods This study recruited 748 children hospitalized for MP pneumonia between January 2021 and October 2023. Patients were classified into two groups: MPP coinfected with respiratory virus group and MPP group. All children underwent polymerase chain reaction testing for respiratory pathogens. Baseline clinical features and demographic data were obtained retrospectively through medical records.

Results The retrospective study included 748 patients, with a viral coinfection rate of 38.75%. Patients in the MPP coinfected with respiratory virus group have a higher disease burden than those in the non-coinfection group. Our findings indicate that patients with Mycoplasma pneumonia co-infected with respiratory viruses had longer hospital stays and prolonged fever post-admission, as well as more severe conditions and a higher incidence of extrapulmonary complications. MPP coinfection was associated with the following factors: patients with extrapulmonary complications of gastroenteritis (OR = 4.474, 95%CI = 1.733–11.554, $P = 0.002$), longer hospital stay (OR = 1.109, 95%CI = 1.012–1.217, $P = 0.027$), longer days of fever after admission (OR = 1.215 95%CI = 1.006–1.469, $P = 0.043$), elevated white blood cell count (OR = 1.332 95%CI = 1.082–1.640, $P = 0.007$), decreased neutrophil count (OR = 0.768 95%CI = 0.602–0.981, $P = 0.035$), higher fibrinogen levels (OR = 1.652 95%CI = 1.138–2.398, $P = 0.008$), and raised lactate dehydrogenase levels (OR = 1.007 95%CI = 1.003–1.011, $P = 0.001$).

Conclusions We determined the clinical significance of respiratory viral coinfection in children with MPP. Timely identification of MPP coinfection and provision of early and comprehensive therapeutic measures are vital in shortening the disease severity and improving prognosis.

Keywords Mycoplasma Pneumoniae Pneumonia, Respiratory virus, Coinfection, Children, Clinical significance

Introduction

Mycoplasma pneumoniae(MP) is a major causative pathogen in community-acquired pneumonia (CAP). Research indicates that MP pneumonia(MPP) accounts for approximately 10–40% of community-acquired pneumonia in children, with higher rates observed during Mycoplasma epidemics [1, 2]. It has been

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reported that about 37.5% of community-acquired pneumonia in children in northern China is caused by MP, occurring in periodic outbreaks every 2–3 years [3]. Generally, most cases of MPP in childhood are benign, presents with mild symptoms and favorable prognosis. However, a significant percentage of MPP instances have been shown to worsen extrapulmonary and intrapulmonary complications [4–8]. Additionally, a large epidemiological study from Taiwan in China demonstrated that patients with Mycoplasma pneumoniae infection have a higher risk of developing early-onset asthma (age < 12 years; aHR, 2.87) and late-onset asthma (age ≥ 12 years; aHR, 3.95) [9], posing great burdens to children's health. Life-threatening pneumonia or even acute respiratory distress syndrome(RDS) requiring extracorporeal membrane oxygen has been reported according to Hsieh's research [10]. Besides, some serious clinical complications such as pleural effusion, lung abscess, atelectasis, necrotizing pneumonia, bronchiolitis obliterans, multi-organ damage, or serious long-term sequelae were associated with severe MPP(SMPP). Regarding pleural effusion, studies have indicated that 64.7% of patients with SMPP admitted to the Pediatric Intensive Care Unit (PICU) experience large pleural effusion (occupying more than half of the chest X-ray) [11]. Furthermore, patients with Mycoplasma pneumonia who have moderate to large pleural effusions (with thoracentesis draining 300–500 mL and ≥ 500 mL of fluid, respectively) exhibit more severe symptoms compared to those without pulmonary complications [12].

Although the exact pathophysiology of SMPP is uncertain, severe and persistent systemic inflammation and MP's resistance to macrolide medications may be crucial factors in the development of SMPP [13, 14]. In recent years, there have been increasing reports of severe pneumonia in children caused by co-infections of Mycoplasma pneumoniae with bacteria (such as Streptococcus pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa) or viruses (including adenovirus, rhinovirus, respiratory syncytial virus, parainfluenza virus, coronavirus, influenza virus, and enterovirus) [15–17]. There were, however, insufficient data on pediatric patients who had MPP coinfected with virus.

To analyze the clinical and laboratory data characterizing MPP and respiratory virus coinfections in MPP and to provide meaningful insight for early intervention and treatment of clinical mycoplasma pneumonia coinfection, this study retrospectively included data from a large sample and underwent strict inclusion and exclusion criteria.

Methods

Patients and groups

The current study was a retrospective analysis that gathered electronic health records from Dandong Central Hospital, China Medical University between January 2021 and October 2023. 738 patients with Mycoplasma pneumoniae pneumonia (MPP) were ultimately enrolled in this study after being evaluated in accordance with the inclusion and exclusion criteria. In our institution, polymerase chain reaction (PCR) testing was a normal procedure for all patients admitted with respiratory tract infections. The existence of respiratory virus coinfection resulted in the division of two groups: the non-coinfection group and the respiratory virus coinfection group.

Diagnosis and definitions

The diagnosed criteria with MPP were according to the Chinese expert consensus on the diagnosis and treatment of Mycoplasma pneumoniae pneumonia in children, version 2023 [18]: (1) respiratory symptoms such as fever, cough, wheezing, dyspnea, and pulmonary rales; (2) typical chest imaging changes (interstitial infiltration, segmental, and lobar consolidation); (3) a positive result for MP-DNA or MP-RNA detected by nucleic acid amplification tests from throat swab specimens; and (4) a single serum MP antibody titer ≥ 1:160, or a fourfold increase in MP antibody titers between two serum samples during the course of illness.

Severe MPP [19] was defined as any of the following criteria (1) Poor general condition or impaired consciousness; (2) Cyanosis or respiratory distress, elevated respiratory rate (≥ 70 /minute in infant, ≥ 50 /minute in older children). (3) Axillary temperature consistently above 39.0 °C for more than 5 days or fever for more than 7 days with no downward trend in peak temperature; (4) Finger pulse oximetry less than 93% on air inhalation at rest; (5) Pulmonary complications such as atelectasis, medium or large pleural effusion(occupying more than half of the chest X-ray) or necrotizing pneumonia; (6) Imaging shows ≥ 2/3 involvement of lung lesions.

Exclusion criteria were as follows (1) age ≤ 1 month or age > 14 years (2) patients with underlying disease including immune deficiencies, respiratory chronic diseases(such as congenital ciliary dyskinesia, diffuse interstitial lung disease, bronchopulmonary dysplasia and bronchial asthma), congenital heart diseases, diabetes, Down's syndrome, epilepsy (3) bacteremia (4) The interval between the two hospitalizations less than 2 months. (5) Referral and discontinuation of treatment or incomplete clinical data. The specific screening process is presented in Fig. 1.

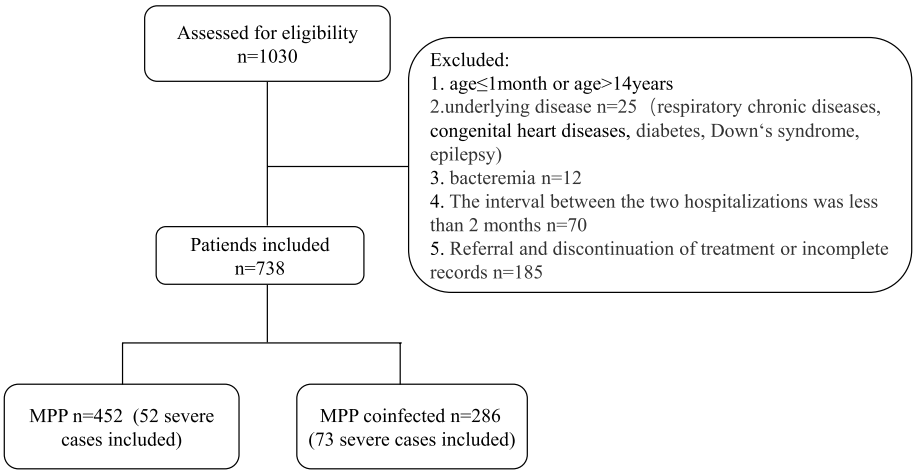

Fig. 1 Flow diagram for the present study

This study was conducted according to the Declaration of 1964 Helsinki and approved by the Ethics Committee of the Dandong Central Hospital. Waiving the requirement for written informed consent due to the retrospective study design and anonymized patient data.

Data collection

Baseline clinical features and demographic data were recorded on the first day of admission. Peripheral blood samples were obtained on patients within 12 h for the determination of laboratory data, such as white blood cell count(WBC), Neutrophil count(N#), lymphocyte count(L#), platelet count(PLT), hemoglobin levels(HB), serum amyloid A(SAA), hypersensitive C-reactive protein(hs_CRP), humoral immunity (including IgA, IgG, IgM), interleukin-6 (IL_6), D-dimer(DD), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase(LDH), total protein(TP), albumin(ALB) and fibrinogen (FIB), etc.

Sample collection

Using commercially available nylon flocked swabs (Mrk Tech, Shenzhen, China), we gently roll the swab over the tonsils and the posterior pharyngeal wall with appropriate pressure, ensuring that contamination from the normal oral flora is avoided. The swab is then placed into 2 mL of Viral Transport Medium (VTM) (Heshengyuan Technology, Qingdao, China), and the Mycoplasma nucleic acids are subsequently detected using Multiplex PCR technology. Simultaneously, two milliliters of peripheral venous blood is centrifuged at 1700×g and 4 °C for 15 min to isolate serum, which is then used to separate and detect Mycoplasma pneumoniae antibodies using the SERODIA MYCO-II kit (Rebio, Tokyo, Japan). It is important to note that all patients included in this study underwent two serological tests for Mycoplasma IgM/IgG during their hospitalization (with a minimum interval of 48 h between the two tests) to reduce selection bias among patients with Mycoplasma pneumoniae [20].

Multiplex-PCR

The swab was then placed into 2 mL of Viral Transport Medium (VTM) (Heshengyuan Technology, Qingdao, China). The VTM was vortexed for 10 s to wash off viruses and virus-containing cells from the oropharyngeal swab. Following the manufacturer's instructions, nucleic acids were extracted using a nucleic acid extraction kit on an automated extraction workstation (Smart LabAssist-16/32) (Healthy Gene Technology Co., Ltd., Ningbo, China).The multiplex PCR was performed as follows: Step 1 involved denaturation at 94 °C for 30 s, followed by a touchdown PCR phase where the temperature was decreased from 65 °C to 60 °C over 30 s, and extension at 72 °C for 1 min, repeated for 6 cycles; Step 2 consisted of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min, repeated for 29 cycles; Step 3 included a final extension at 72 °C for 10 min; and Step 4 concluded with holding at 4 °C. The 10 μL amplified products were added to 287 μL loading buffer and 3 μL SizeStandard-400, and subsequently analyzed using the GenomeLab GeXP Genetic Analysis System.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Version 25.0. A P value < 0.05 was considered statistically significant. Continuous variables were expressed as the means ± standard deviations (SD) or median with interquartile range (IQR). Normally distributed data were compared using the independent-samples Student's t-test, while skewed data were compared using the Mann–Whitney U test. Categorical variables were

expressed as frequencies and percentages. The chi-square test was used to compare groups. The logistic regression model was used to analyze the risk of other comorbidities in groups. Age and gender were adjusted as covariates to eliminate the potential statistical errors on univariate variables and the variables with covariates showing statistical significance were included in the multivariate logistic regression analysis to further identify the association with respiratory virus coinfection in MPP patients.

Results

General data of patients

A total of 738 children aged 31 days to 14 years were eventually enrolled in the study with an average age of 7.08 ± 2.76 years old. Of these, 452 patients were in the MPP group (52 patients were diagnosed with SMPP), and 286 patients were in the MPP combined with the respiratory virus group (73 patients were diagnosed with SMPP). The selection process is illustrated in Fig. 1.

Table 1 presents the baseline demographic and clinical characteristics of the two groups. Children in the MPP group were older than those in the respiratory virus coinfection group (7.46 ± 2.67 years vs. 6.49 ± 2.80 years, $P < 0.001$). The proportion of males was significantly higher in the MPP coinfection group. Factors including total duration of hospital stay, peak fever before hospitalization, days of fever after admission, and proportion of severe cases were significantly longer or higher in patients with the MPP coinfection group compared to the MPP group ($p < 0.05$). In addition, the proportion of extrapulmonary complications, specifically rash and gastroenteritis, was significantly higher in the MPP coinfection group; however, no significant differences were observed in myocardial injury, hypokalemia, otitis media, or liver dysfunction between the two groups.

Table 1 Comparison of general data and clinical characteristics between the two groups

Variables	MPP group (*N* = 452)	MPP coinfection group (*N* = 286)	*p*
age, years	7.46 ± 2.67	6.49 ± 2.80	< 0.001
sex. n(%)			0.017
Female	240(53.1)	126(44.1)	
Male	212(46.9)	160(55.9)	
Days of hospital stay	8.75 ± 3.14	9.73 ± 3.49	< 0.001
Cough days before admission	7.65 ± 5.29	7.79 ± 4.73	0.710
Peak fever before admission	39.03 ± 0.86	39.17 ± 0.82	0.028
Fever days after admission	1.21 ± 1.56	1.49 ± 1.67	0.025
rale. n(%)			0.186
No	140 (31)	102 (35.7)	
Yes	312 (69)	184 (64.3)	
severity. n(%)			< 0.001
mild	400 (88.5)	213 (74.5)	
severe	52 (11.5)	73 (25.5)	
Extra-pulmonary complications. n(%)	68(15.0)	85(29.7)	
rash	5 (1.1)	10 (3.5)	0.025
gastroenteritis	16 (3.5)	33 (11.5)	< 0.001
Myocardial injury	14 (3.1)	16 (5.6)	0.094
Hypokalemia	13 (2.9)	7 (2.4)	0.727
otitis media	3 (0.7)	4 (1.4)	0.316
Liver dysfunction	17 (3.8)	15 (5.2)	0.335

Continuous variables were expressed as the means ± standard deviations (SD) or median with interquartile range (IQR), Categorical variables were expressed as frequencies and percentages, $P < 0.05$ was considered statistically significant

Distribution and prevalence of respiratory virus

286 patients had respiratory virus coinfection in this study. The overall coinfection rate was 38.75% (286/738). Among them, 73 patients developed into severe cases higher than those in the MPP group (25.5% vs 11.5%, $P < 0.001$). Adenovirus was the most prevalent organism accounting for 43.4% (124/286), followed by Rhinovirus (36.0%, 103/286) (Fig. 2). A total of 41 (14.3%) patients were detected with more than two co-infections.

Laboratory findings of patients in two groups

As shown in Table 2, we found that significant differences in WBC, N#, RDW, PLT, SAA, DD, FIB, IgM, Cr, ALT, LDH, TP, Lpa, CK between the two groups ($P < 0.05$); however, no remarkable differences were observed in L#, HB, hs_CRP, IL_6, IgA, IgG, AST, ALB, CKMB. Furthermore, levels of WBC, N#, RDW, PLT, SAA, FIB, IgM, and LDH levels were significantly higher among patients in the MPP coinfection group compared to those in the MPP group ($P < 0.05$).

Factors associated with respiratory virus coinfection in MPP patients

After adjusting for age and sex as covariates, the result showed that MPP patients with respiratory virus coinfection were associated with gastroenteritis, rash, longer duration of hospital stay, peak fever before hospitalization, and days of fever after admission. Similar results were observed regarding laboratory features such as WBC, N#, RDW, PLT, SAA, FIB, IgM, and LDH (Table 3). Additionally, multivariable logistic regression was performed to further verify the associations between these variables and MPP coinfection. MPP coinfection was associated with the following factors: extrapulmonary complications of gastroenteritis in patients, prolonged hospital stay, extended duration of fever after admission, elevated white blood cell count, decreased neutrophil count, higher fibrinogen levels, and raised lactate dehydrogenase levels.

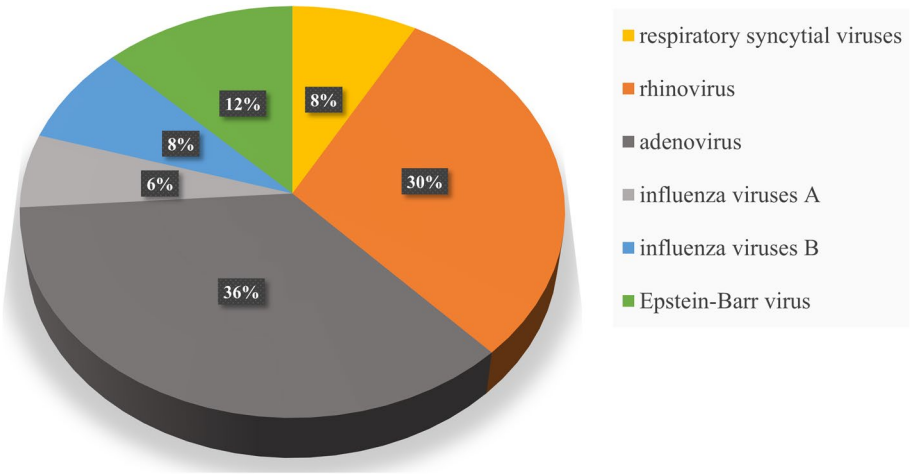

Fig. 2 Distribution of respiratory virus

Table 2 Comparison of Laboratory data between the two groups

Variables	MPP group (*N* = 452)	MPP coinfection group (*N* = 286)	*P*
WBC, × 10^9/L	7.68(6.10,9.85)	9.17(6.65,12.14)	< 0.001
N#, × 10^9/L	4.91(3.81,6.49)	5.75(3.85,8.19)	< 0.001
L#, × 10^9/L	1.95(1.45,2.59)	2.1(1.40,4.18)	0.094
HB, g/L	125(120,132)	126(119,132)	0.785
RDW, %	12.9(12.0,13.4)	13.0(12.6,13.7)	0.002
PLT, × 10^9/L	276(235,343)	304(247,367)	0.002
SAA, mg/L	58.36(23.38,144.07)	79.45(32.73,168.45)	0.016
Hs_CRP, mg/L	11.17(4.46,20.76)	11.58(4.88,24.00)	0.490
DD, ug/ml	0.45(0.32,0.64)	0.39(0.25,0.63)	0.002
FIB, g/L	4.11(3.70,4.70)	4.27(3.80,4.87)	0.037
IL-6, pg/mL	13.81(7.73,24.15)	13.95(6.97,23.86)	0.955
IgA, g/L	1.16(0.87,1.61)	1.25(0.94,1.67)	0.176
IgM, g/L	1.24(1.01,1.60)	1.38(1.04,1.74)	0.041
IgG, g/L	10.5(8.96,12.48)	10.5(9.07,11.60)	0.475
Cr, umol/L	37(31,42)	33(28,40)	< 0.001
ALT, U/L	14(11.19)	13(11,18)	0.039
AST, U/L	26(23,32)	27(23.33)	0.396
LDH, U/L	278(252,321)	319(265,380)	< 0.001
TP, g/L	69(65,72)	66(62,70)	< 0.001
ALB, g/L	43(41,45)	43(41,45)	0.976
Lpa, mg/L	90(46,183)	56(7,157)	< 0.001
CKMB, U/L	17(14,23)	17(14,23)	0.414
CK, U/L	79(52,115)	67(44,104)	< 0.001

WBC white blood cells, N# neutrophils, L# lymphocytes, Hb hemoglobin, RDW red blood cell distribution width, PLT platelet, SAA serum amyloid A, Hs_CRP hypersensitive C-reactive protein, DD D-dimer, FIB fibrinogen, IL-6 interleukin 6, IgA, IgG, IgM humoral immunity A, G and M, Cr creatinine, AST glutamic oxaloacetic transaminase, ALT glutamic-pyruvic transaminase, TP total protein, ALB albumin, Lpa lipoprotein a, CK creatine kinase, CK-MB creatine kinase-MB, $P < 0.05$ was considered statistically significant

Discussion

MP is one of the most common pathogens in children with CAP. The proportion of MP coinfected with respiratory virus is generally increasing [15–17, 21, 22]. This proportion changes with geographical region, study population, and season of onset [23]. We identified that higher levels of white blood cells (WBC), fibrinogen (FIB), and lactate dehydrogenase (LDH), decreased neutrophil count (N#), prolonged duration of fever after admission, extended hospital stay, and the proportion of gastroenteritis were associated with respiratory viral coinfection in children hospitalized with MP pneumonia. In addition, we found that such co-infections increase the likelihood of SMPP.

MPP can occur at any age, especially in preschool and school children. Children in the coinfected group were significantly younger than those in the non-coinfection group, consistent with the results of previous studies [24, 25]. Similarly, there is a notable difference in the gender distribution between the two groups. We found that the proportion of females was higher in the MPP group compared to males; however, males were more likely to be in the coinfected group in our study. Evidence suggests that prepubescent boys have shorter and narrower airways than girls, potentially increasing their susceptibility to viral infections and the risks of lower respiratory tract infections and delayed pathogen clearance [26, 27]. Additionally, males generally exhibit weaker innate and adaptive immune responses, accumulate higher viral loads, and require longer times to clear viruses compared to females [28]. Some studies, however, did not find a statistically significant gender difference between the MPP and MPP coinfection groups [21, 22]. Given the complex relationship between the observed gender distribution and

Table 3 Factors associated with respiratory virus coinfection in MPP

Variables	OR95%CI	P#	OR95%CI	P*
gastroenteritis	3.907 (2.083–7.327)	0.000	4.474(1.733–11.5454)	0.002
rash	3.318 (1.105–9.966)	0.033		
severe	2.602 (1.744–3.883)	0.001		
Days of hospital stay	1.099 (1.049–1.152)	0.001	1.109(1.012–1.217)	0.027
Peak fever before admission	1.319 (1.095–1.590)	0.004		
Fever days after admission	1.122 (1.019–1.235)	0.019	1.215(1.006–1.469)	0.043
WBC	1.073 (1.030–1.118)	0.001	1.332(1.082–1.640)	0.007
N#	1.087 (1.033–1.144)	0.001	0.768(0.602–0.981)	0.035
RDW	1.260 (1.022–1.554)	0.030		
PLT	1.002 (1.000–1.004)	0.019		
SAA	1.002 (1.000–1.004)	0.010		
FIB	1.414 (1.156–1.729)	0.001	1.652(1.138–2.398)	0.008
IgM	1.413 (1.068–1.870)	0.016		
LDH	1.006 (1.003–1.008)	0.001	1.007(1.003–1.011)	0.001

WBC white blood cells, N# neutrophils, RDW red blood cell distribution width, PLT platelet, SAA serum amyloid A, FIB fibrinogen, LDH L-lactate dehydrogenase, IgM humoral immunity A, $P < 0.05$ was considered statistically significant

[#] significant variables after adjusted by age and sex

[*] significant variables in multivariable logistic regression analysis

infection status, we suggest that future research could explore the roles of different genders in MPP and respiratory viral co-infections, while considering factors such as individual hormone secretion levels, social behaviors, and lifestyle influences.

Adenovirus was the most frequently detected pathogen with MPP in our study. The clinical manifestations of adenovirus infections in children are varied and include ocular, respiratory, and gastrointestinal symptoms [29, 30]. Studies have indicated that children infected with adenovirus type 3(AdV-3) experience more lower respiratory tract infections, more severe gastrointestinal symptoms, and higher hospitalization rates compared to those infected with adenovirus type 2(AdV-2) [31]. It's reported that adenovirus and MP were the most common pathogens of co-infection in hospitalized children [32]. Furthermore, the co-infection of the two viruses was associated with a more severe illness [33, 34]. Gao J et al. reported that children with MPP coinfected with adenovirus had longer hospital stays and febrile course, higher incidence of respiratory distress, severe pneumonia, and oxygen therapy [35]. Co-infection with MP and adenovirus can elicit a heightened immune response in the body, whereby the release of cytokines induces an inflammatory response that leads to damage to intestinal epithelial cells and increased intestinal permeability. This may allow undigested food components and toxins to permeate the body, resulting in gastrointestinal symptoms such as abdominal pain and diarrhea. In our study, the proportion of patients experiencing gastrointestinal symptoms and severe pneumonia in the mycoplasma co-infection group was higher than that in the mycoplasma-only group.

In our investigation, respiratory virus coinfection was linked to longer and more severe illness courses in MPP patients. Patients with MPP co-infection experienced a longer duration of fever after admission compared to those with mycoplasma infection alone. Continued fever in children after hospitalization typically indicates an excessively strong immune-inflammatory response, which may lead to systemic immune dysfunction, making it difficult to combat infections and control inflammation. This can further result in extended duration of fever and lengthened hospital stays [36]. If the systemic immune dysfunction and excessive inflammatory response are not addressed, it may lead to severe or refractory MPP [37–39]. Similar results were seen in Zhou et al. [21], reporting that a higher risk of pulmonary consolidation was associated with adenovirus coinfection with MPP. According to Choo et al. [24], respiratory virus coinfection in MPP was associated with a longer duration of fever, severe pneumonia, and poor response to the treatment for MPP. Virus coinfection may play an important role in the development of MP necrotizing pneumonia according to Hsieh Y-C et al. [40]. However, no significant differences were observed in terms of clinical course and prognosis between the patients infected with either MP alone or with respiratory virus coinfection according to Chiu et al. [41]. and Zhao et al. [22]. This finding may be attributed to the diversity of research populations

or the seasonal prevalence of respiratory viruses, which requires further exploration in future studies.

White blood cells, particularly neutrophils, are essential immune cells in the human body. Neutrophil infiltration is recognized as a characteristic feature of Mycoplasma pneumonia and is an important factor influencing the progression of MPP [42, 43]. The rapid recruitment of neutrophils to infected tissues is crucial for the innate immune system. Research indicates that IL-8 is the most effective neutrophil inducer for chemotaxis to sites of infection [44]. Furthermore, IL-8 promotes the release of matrix metalloproteinase-9 (MMP-9), myeloperoxidase (MPO), neutrophil elastase (NE), and neutrophil extracellular traps (NETs) to capture and kill pathogens [45, 46]. However, overactivation of neutrophils can result in immune dysfunction and contribute to the development of severe pneumonia [42]. In our current study, after we included factors that were statistically significant after adjusting for sex and age in the multivariate logistic regression, we found that the odds ratio (OR) for neutrophils decreased. This is because neutrophil counts are influenced by various factors, including the degree of inflammatory response, the individual's immune status, and the type of infection. In regression analyses, other potential confounding factors may diminish the impact of neutrophil counts. Therefore, while an increase in neutrophil counts was observed in the overall sample, its predictive capability regarding specific infection risk may be limited. This indicates the need to further integrate biomarkers that may influence disease outcomes in future studies to explore interactions among other variables and enhance the ability to predict MPP co-infection.

Lactate dehydrogenase (LDH), as well, has long been regarded as a nonspecific inflammatory factor and reliable evaluation index of disease severity. It mainly exists in red blood cells, myocardium, liver, kidney, skeletal muscle, and lung tissues. When pathogens invade the host organism causing inflammatory response and damage to tissue cells, LDH is released into the bloodstream resulting in increased LDH. Similarly, LDH levels are higher in severe cases of MPP than in mild cases [12, 47]. Moreover, elevated LDH levels may be associated with necrotizing pneumonia associated with Mycoplasma pneumoniae [48]. Choo et al. also reported that LDH was significantly higher in the respiratory virus coinfection group in MPP patients than in the non-coinfection group [24]. Our present study revealed that higher LDH levels are associated with disease severity and can be seen as a significant biomarker of MPP coinfection, which is consistent with previous reports.

Fibrinogen (FIB) is known as one of the acute phase proteins primarily synthesized in hepatocytes, which is involved in fibrin formation as a trigger for coagulation activity. During the acute phase of the inflammatory response, the expression and synthesis of FIB increased and correlate strongly with disease severity. The level of fibrinogen was found to be higher in critical COVID-19 patients compared to those with mild or moderate cases [49, 50]. In the present study, FIB levels were significantly elevated in MPP patients coinfected with respiratory virus, and same results were observed after adjusting for potential covariates. In multiple logistical regression analysis, FIB was identified as a valuable indicator for distinguishing between MPP and MPP coinfection.

These findings suggest that respiratory virus coinfection exacerbates clinical severity, with the heightened immune response potentially contributing to the development of SMMP. Each disease involves pathogenic or inflammation-inducing substances, and the host's immune system responds not only to pathogens but also to inflammatory mediators derived from them. To better elucidate the pathophysiological mechanisms of these diseases, some studies have proposed the Protein Homeostasis System (PHS) hypothesis [51–53]. This hypothesis posits the existence of a system that regulates all biological activities within the organism, including immune responses. All biological responses observed during Mycoplasma and respiratory virus infections in hosts—such as the laboratory indicators and extrapulmonary complications noted in the co-infection group in this study—may be controlled by the body's integrated conversion control system. Research has indicated that Mycoplasma co-infection may further worsen disease severity through direct tissue damage or indirect immune responses [33]. Therefore, early treatment with appropriate doses of immunomodulators (corticosteroids) for MP infection and related immune-mediated diseases is crucial [52, 54].

Limitations

This study was a retrospective study and a multi-center study is needed in the future. Second. it is important to recognize that variations in positive diagnostic results may arise due to differences in Mycoplasma pneumoniae detection methods, serological kits, and other uncontrollable factors. This underscores the necessity for careful consideration and further investigation to enhance the accuracy and reliability of diagnostic procedures in future research. We regret that due to current limitations of our hospital's laboratory testing equipment, we are unable to definitively identify patients with pathogen colonization. We hope that future research will elucidate the impact of pathogen colonization based on our findings. However, despite these limitations, the present findings are still meaningful since respiratory virus coinfection in MPP patients is not uncommonly observed.

Conclusions

In the study, we determined the clinical significance of respiratory viral coinfection in children with MPP. We found that MPP coinfection was significantly associated with various clinical and laboratory indices and may contribute to the development of SMPP. Furthermore, this research is significant as it contributes to understanding the pathophysiology and could influence future treatment strategies for MPP.

Abbreviations

MP	Mycoplasma Pneumoniae
MPP	Mycoplasma Pneumoniae Pneumonia
SMPP	Severe Mycoplasma Pneumoniae Pneumonia
CAP	Community-acquired pneumonia
NPSs	Nasopharyngeal swabs

Acknowledgements

The authors wish to thank patients and participating investigators associated with the clinical studies.

Authors' contributions

Study concept: TF and ASY. Study design: All authors. Acquisition, analysis, or interpretation of data: ASY, LYR, and XJS. Statistical analysis: ASY. Drafting of the manuscript: ASY. Critical revision of the manuscript for important intellectual content: All authors.

Funding

Not applicable.

Data availability

All the data used and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study adhered to the ethical principles outlined in the 1964 Helsinki Declaration and its subsequent amendments. Approval for the study was obtained from the Ethics Committee of Dandong Central Hospital (Approval No. DDZX-20240517), and it was determined that written informed consent was not required, as per the committee's exemption.

Consent for publication

Not Applicable.

Competing interests

The authors declare no competing interests.

Received: 18 May 2024 Accepted: 4 November 2024
Published online: 25 November 2024

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