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ORIGINAL RESEARCH

Outcomes of *M. pneumoniae* pneumonia with mixed infection

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Abstract

Background: *Mycoplasma pneumoniae* pneumonia (MPP) is often complicated with mixed infections that worsen the prognosis, but the outcomes in pediatric cases are unclear. The aim of this study is to investigate the association of mixed infection and outcomes in severe MPP that occurs in childhood. **Methods**: This retrospective study included 184 pediatric cases of severe MPP that were managed at our hospital (between January 2014 and December 2017). The cohort was divided into the single *Mycoplasma pneumoniae* infection, mixed infection with a noxa other than *M. pneumoniae*, and mixed infection with two or more noxae other than *M. pneumoniae* groups. The demographic and clinical information of the patients was compared via statistical analysis. **Results**: The incidence of mixed infections was high at 64.1%. Cytomegalovirus and Epstein-Barr virus were the most common causes of mixed infection. According to the findings of binary logistic regression analysis, the presence of more than one pathogen (other than *M. pneumoniae*) was positively associated with the score determined from Pediatric Risk of Mortality III (β = 0.760, odds ratio [OR] = 2.139, 95% confidence interval [CI] = 1.391-2.390, P = 0.001), Pediatric Critical Illness Score (β = 1.203, OR = 3.328, 95% CI = 1.723-6.731, P = 0.000), and total length of hospital stay (β = 0.730, OR = 2.075, 95% CI = 1.404-3.066, P = 0.000). **Conclusion**: Viral and bacterial mixed infection in pediatric cases of severe MPP is positively associated with hospitalization period and disease severity, and ultimately, may increase the chances of severe illness and death among children.

Keywords

Mycoplasma pneumonia; Severity; Mixed infection; PRISM III; PCIS; Retrospective

1. Background

Recently, the incidence of *Mycoplasma pneumoniae* infection has been found to gradually be increasing. *M. pneumoniae* has been reported to be a common cause of community-acquired pneumonia (CAP) in older children [1–3]. This finding has important implications, as CAP is a principal reason for morbidity and mortality worldwide [4]. In addition to the high incidence of this pathogen, mixed infection in common in children with *M. pneumoniae* pneumonia (MPP). A retrospective descriptive study of 10,039 consecutive children with MPP showed that 2% of them were also infected with another bacterial pathogen [5]. Another study of 286 children with MPP showed that 84 (29.4%) had a mixed infection with a respiratory virus, and the highest co-detection rate was observed in young children [6]. Li *et al*. reported a case of mixed infection with Epstein-Barr virus, cytomegalovirus, and *M. pneumoniae*; their case exemplifies the challenges in the diagnosis of mixed infection with multiple respiratory pathogens and the associated complications [7]. With regard to the prognosis of MPP accompanied by mixed infection, a study on adults showed that bacterial mixed infection with a respiratory virus may worsen patient outcome, including the severity and mortality of CAP [8]. However, there is no consensus about the prognosis of mixed infections in children with MPP. For example, a prospective study of 59 children demonstrated that the clinical features, complications, and outcomes of patients with *M. pneumoniae* infection was not significantly differently from those of patients who had mixed infection with *M. pneumoniae* and a viral pathogen [9], while a retrospective study of 396 children with refractory MPP showed that mixed infection was associated with higher disease severity than single infections were [10]. Given these contradictory findings, it is important to investigate this topic further, in order to improve the diagnosis and prognosis of mixed infection with MPP and other pathogens in children.

The presence of mixed infections in children who have severe MPP has not received much attention. Therefore, in order to shed light on the prognosis of this patient group, the present retrospective research was based on the hypothesis that in children, mixed infections are associated with the degree of severity and the disease course of MPP.

TABLE 1. Characteristics of the study cohort (N = 184).

Characteristics	Single *M. pneumoniae* infection (n = 66)	Mixed infection of a noxa other than *M. pneumoniae* (n = 58)	Mixed infection with two or more noxae other than *M. pneumoniae* (n = 60)	F/Z/χ^2	*P* value
Gender (*n*, % males)	44 (66.7%)	40 (69.0%)	38 (63.3%)	0.425	0.809
Age (mo)	24.59 (6.25-36.00)	23.39 (7.38-36.00)	23.02 (4.00-30.00)	0.143	0.931
Prematurity (< 37 weeks GA) (*n*, %)	7 (10.6%)	5 (8.6%)	6 (10.0%)	0.143	0.931
Maximum temperature > 39 °C (*n*, %)	24 (36.4%)	20 (34.5%)	30 (50.0%)	3.589	0.166
Length of fever > 7 d (*n*, %)	9 (13.6%)	12 (20.1%)	16 (26.7%)	3.339	0.188
Length of fever > 10 d (*n*, %)	7 (10.6%)	8 (13.8%)	10 (16.7%)	0.986	0.611
Non-invasive ventilation (*n*, %)	26 (39.4%)	28 (48.3%)	19 (31.7%)	2.929	0.231
Invasive mechanical Ventilation (*n*, %)	9 (13.6%)	6 (10.3%)	19 (31.7%)	11.325	0.003
Respiratory failure (*n*, %)	44 (66.7%)	36 (62.1%)	35 (58.3%)	0.938	0.626
Pleural effusion (*n*, %)	3 (4.5%)	3 (5.2%)	9 (15.0%)	5.592	0.061
Interstitial lung disease (*n*, %)	1 (1.5%)	1 (1.7%)	3 (5.0%)	1.76	0.415
Heart failure (*n*, %)	4 (6.1%)	4 (6.8%)	13 (21.7%)	9.28	0.01
Arrhythmia (*n*, %)	3 (4.5%)	2 (3.4%)	2 (3.3%)	0.156	0.925
Myocardiopathy (*n*, %)	2 (3.0%)	1 (1.7%)	2 (3.3%)	0.318	0.853
Myocarditis (*n*, %)	1 (1.5%)	2 (3.4%)	1 (1.7%)	0.68	0.712
Hydropericardium (*n*, %)	1 (1.5%)	2 (3.4%)	3 (5.0%)	1.285	0.526
Kawasaki disease (*n*, %)	1 (1.5%)	1 (1.7%)	2 (3.3%)	0.598	0.741
Myocardial damage (*n*, %)	10 (15.2)	8 (13.8%)	7 (11.67%)	0.262	0.877
Extrapulmonary complications of two or more systems (*n*, %)	24 (36.4%)	21 (36.2%)	33 (55.0%)	6.219	0.045
Total length of stay in hospital (d)	11.32 (7.00 - 13.50)	13.00 (7.00 - 14.00)	21.76 (10.00 - 25.00)	25.452	0
Total length of stay in hospital > 14 d (*n*, %)	16 (24.2%)	15 (25.8%)	34 (56.7%)	18.455	0
PCIS < 80 (*n*, %)	3 (4.5%)	4 (6.9%)	17 (28.3%)	18.501	0
PRISM III scores	1.97 (0 - 3)	2.10 (0 - 3)	5.57 (0 - 9.75)	10.654	0.005
PRISM III estimated mortality (%)	1.46 (0.78 - 1.49)	1.61 (0.76 - 1.51)	8.93 (0.78 - 5.68)	9.525	0.009
White blood cell count ($\times 10^9$/L)	13.71 ± 6.81	13.44 ± 6.25	16.11 ± 7.61	2.683	0.071
Hemoglobin (g/L)	116.21 ± 20.37	113.79 ± 18.45	109.45 ± 20.13	1.883	0.155
Platelet count ($\times 10^9$/L)	404.00 ± 162.55	408.76 ± 202.84	357.85 ± 197.31	1.357	0.26
Red blood cell volume distribution width (%)	13.91 ± 2.42	14.47 ± 1.63	14.00 ± 2.50	1.09	0.338
C-reactive protein (mg/L)	31.11 (0.55 - 31.85)	22.83 (0.50 - 33.17)	55.16 (3.33 - 69.56)	5.473	0.065
Pre-albumin (mg/dL)	15.58 (12.99 - 18.12)	15.20 (10.22 - 18.48)	14.94 (10.18 - 18.66)	2.277	0.32
Procalcitonin (ng/L)	0.75 (0.01 - 22.46)	0.18 (0.05 - 3.64)	0.27 (0.04 - 4.07)	2.356	0.308
Lactate dehydrogenase (U/L)	723.06 (323.70 - 860.00)	753.36 (331.48 - 973.35)	962.30 (339.23 - 870.85)	0.458	0.795
d-dimer (mg/L)	1.42 (0.32 - 1.11)	1.98 (0.34 - 1.93)	5.59 (0.50 - 3.62)	13.277	0.001
Co-infection with bacteria	0	10	13	15.23	0

2. Materials and methods

2.1 Study population

This retrospective research was conducted over a 48-month period (January 2014 to December 2017) and included children with severe MPP at a tertiary hospital in Fujian Province, P. R. China. *M. pneumoniae* infection was diagnosed based on positive serological results for total antibody (≥ 1 : 640), IgM antibody and related clinical manifestations (dry cough, fever, and radiological pulmonary abnormalities). The severity of MPP was determined according to standard guidelines [11] (see the Supplementary material).

TABLE 2. Identified pathogens.

Pathogens	N = 200
Mixed **infection with a noxa**	**58 (49.2%)**
Virus	**48 (40.7%)**
Cytomegalovirus (CMV)	11 (9.3%)
The data of viral load of CMV: 2.14 × 10^4/mL, 3.66 × 10^4/mL, 2.86 × 10^4/mL, 5.39 × 10^5/mL, 2.01 × 10^5/mL, 6.13 × 10^4/mL, 4.33 × 10^6/mL, 1.01 × 10^4/mL, 9.08 × 10^5/mL, 3.72 × 10^5/mL, 2.30 × 10^4/mL	
Epstein-Barr virus (EB)	9 (7.6%)
The data of viral load of EB: 4.92 × 10^6/mL, 4.18 × 10^7/mL, 1.59 × 10^5/mL, 2.09 × 10^4/mL, 6.23 × 10^6/mL, 7.12 × 10^5/mL,4.15 × 10^6/mL, 6.16 × 10^4/mL, 7.89 × 10^5/mL	
Chlamydia pneumoniae (Cp)	8 (6.8%)
Influenza B	4 (3.4%)
Respiratory syncytial virus (RSV)	4 (3.4%)
Enterovirus type 71 (EV71)	3 (2.5%)
Influenza A	2 (1.7%)
Rotavirus (RV)	2 (1.7%)
Adenovirus (ADV)	1 (0.8%)
Parainfluenza viruses (PV)	1 (0.8%)
Astrovirus (AV)	1 (0.8%)
Herpes Simplex virus (HSV)	1 (0.8%)
Rubellavirus (RuV)	1 (0.8%)
Bacteria	**10 (8.5%)**
Klebsiella pneumoniae	2 (1.7%)
Pseudomonas aeruginosa (PsAr)	2 (1.7%)
Staphylococcus aureus (SA)	1 (0.8%)
Legionella pneumophila (LP)	1 (0.8%)
Haemophilus influenzae	1 (0.8%)
Acinetobacter baumannii (AB)	1 (0.8%)
Escherichia coli (EC)	1 (0.8%)
Streptococcus pneumoniae (Sp)	1 (0.8%)
Mixed **infection with two or more noxae** (The data of viral load of CMV/EB)	**60 (50.8%)**
CMV + Influenza B (2.22 × 10^4/mL, 2.67 × 10^5/mL, 5.16 × 10^6/mL, 3.12 × 10^6/mL, 2.14 × 10^5/mL)	5 (4.2%)
EB + HSV (3.33 × 10^6/mL, 6.04 × 10^5/mL, 4.13 × 10^7/mL, 7.04 × 10^6/mL)	4 (3.4%)
EB + Influenza B (5.41 × 10^5/mL, 4.22 × 10^5/mL, 9.63 × 10^6/mL)	3 (2.5%)
CMV + Cp (2.46 × 10^5/mL, 2.12 × 10^6/mL, 4.44 × 10^6/mL)	3 (2.5%)
CMV + ADV (1.23 × 10^6/mL, 2.50 × 10^5/mL)	2 (1.7%)
CMV + EC (3.18 × 10^4/mL, 7.56 × 10^5/mL)	2 (1.7%)
CMV + *Candida albicans* (1.23 × 10^6/mL, 4.91 × 10^5/mL)	2 (1.7%)
EB + EV71 (3.29 × 10^5/mL, 5.05 × 10^6/mL)	2 (1.7%)
EB + Influenza A (3.03 × 10^5/mL, 6.43 × 10^5/mL)	2 (1.7%)
Cp + HSV	2 (1.7%)
CMV + SA (2.22 × 10^5/mL)	1 (0.8%)
CMV + EV71 (2.43 × 10^5/mL)	1 (0.8%)
EB + RuV (1.67 × 10^7/mL)	1 (0.8%)
EB + *Candida albicans* (3.66 × 10^5/mL)	1 (0.8%)
EB + RSV (4.91 × 10^4/mL)	1 (0.8%)
EB + RV (2.18 × 10^5/mL)	1 (0.8%)
Cp + LP	1 (0.8%)
Cp + RSV	1 (0.8%)
SA + ADV	1 (0.8%)
SA + AB	1 (0.8%)
AB + *Klebsiella pneumoniae*	1 (0.8%)
AB + *Enterobacter cloacae*	1 (0.8%)
Candida albicans + SA	1 (0.8%)
Candida albicans + PV	1 (0.8%)
Candida albicans + EV71	1 (0.8%)
Candida albicans + AB	1 (0.8%)
Candida albicans + Sp	1 (0.8%)
Candida albicans + *Stenotrophomonas maltophilia*	1 (0.8%)
EB + EV71 + Cp (1.65 × 10^7/mL, 2.57 × 10^5/mL)	2 (1.7%)
AB + PsAr + *Klebsiella pneumoniae*	2 (1.7%)
CMV + HSV + EC (5.06 × 10^6/mL)	1 (0.8%)
CMV + Cp + RSV (4.17 × 10^5/mL)	1 (0.8%)
CMV + HSV + Aspergillus (1.21 × 10^6/mL)	1 (0.8%)
EB + Cp + Aspergillus (1.97 × 10^6/mL)	1 (0.8%)
EB + Influenza A + EV71 (3.54 × 10^5/mL)	1 (0.8%)
CMV + ADV + *Stenotrophomonas maltophilia* (1.61 × 10^6/mL)	1 (0.8%)
CMV + *Stenotrophomonas maltophilia* + PsAr (9.05 × 10^6/mL)	1 (0.8%)
EB + CMV + *Candida albicans* + EC (EB:9.69 × 10^6/mL ; CMV: 8.31 × 10^6/mL)	1 (0.8%)
CMV + RSV + *Haemophilus influenza* + *Aspergillus* (1.23 × 10^5/mL)	1 (0.8%)
EB + CMV + HSV + LP + PsAr (EB: 7.14 ×10^6/mL; CMV: 1.65 ×10^5/mL)	1 (0.8%)
EB + CMV + HSV + LP + RSV + PV (EB: 6.21 × 10^6/mL ; CMV: 4.15 ×10^6/mL)	1 (0.8%)

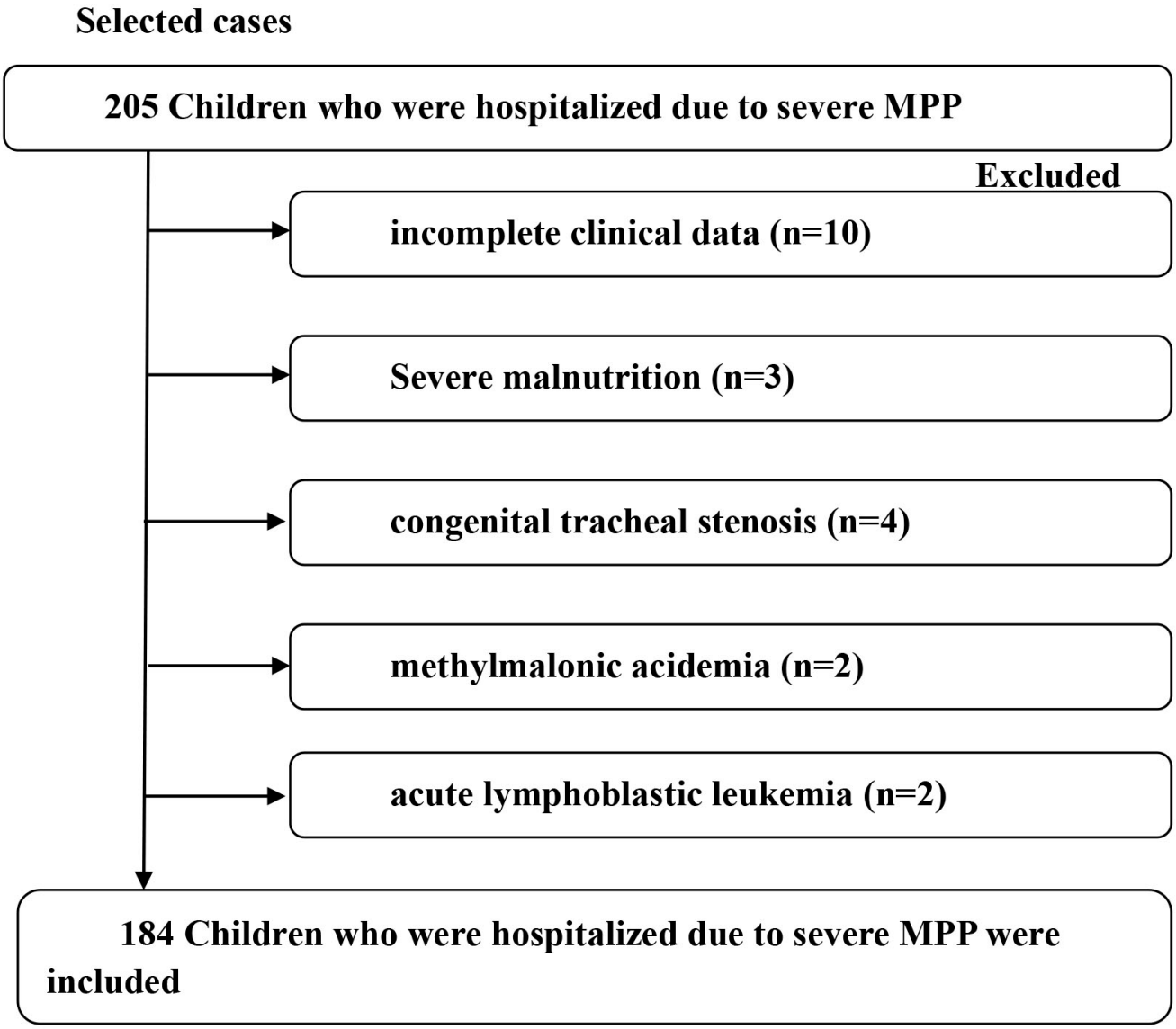

FIGURE 1. Flow chart showing the selection and exclusion of the patient population in this study.

The exclusion criteria were abnormal development of the airway, presence of a congenital disease, presence of a genetically transmitted metabolic or autoimmune disease, presence of a neoplastic disease, malnutrition, human immunodeficiency virus infection, tuberculous infection, and incomplete clinical data.

2.2 Demographic and clinical data

The following information was collected from the medical records of the included patients: (1) basic demographic and clinical data (age, gender, hospitalization time, provision of supplemental oxygen, and duration of fever); (2) the data from laboratory tests performed within 24 h of admission (C-reactive protein [CRP] level, D-dimer levels, hemoglobin [Hb] level, lactate dehydrogenase [LDH] level, platelet [PLT] count, pre-albumin [PA] level, procalcitonin [PCT] level, red blood cell volume distribution width [RDW], and white blood cell [WBC] count), and the data from blood culture exam, chest radiography, and electrocardiography; (3) data on indicators of the degree of disease severity (systolic blood pressure, temperature, mental health status, status of the gastrointestinal system, heart beat rate, pupillary reflexes, glucose level, sodium level, potassium level, creatinine level, urea level, Hb level, PLT count, prothrombin time or partial thromboplastin time, WBC count, acidosis [pH], total CO_2, PCO_2, and arterial PaO_2). Based on the worst values of the indicators of disease severity that were obtained within 24 h after hospital admission, the Pediatric Risk of Mortality (PRISM) III score (higher PRISM III score levels being associated with increased estimated mortality) and Pediatric Critical Illness Score (PCIS) were calculated. Within 72 h after admission, serum samples from the patients were examined for co-infection with the help of blood culture and deep sputum cultures for bacterial infection (a positive response was defined as two consecutive positive results for the same bacterial species), passive agglutination test for antibodies against *M pneumonia* (FUJIREBIO INC, Japan), indirect fluorescence test for antibodies against nine noxaes that are commonly implicated in co-infection (ade-

novirus, Chlamydia pneumoniae, Coxiella burnetti, influenza viruses A and B, Legionella pneumophila, M. pneumoniae, parainfluenza viruses 1, 2, and 3, and respiratory syncytial virus) (VIRCELL.S.L PNEUMOSLIDE IgM,Spain), fluorometric PCR assay for Epstein-Barr virus and cytomegalovirus (Sun Yat-sen University Daan Gene C, Ltd . P. R. China), colloidal gold technique for enterovirus type 71 (Beijing Wantai Biological Pharmaceutical Co, Ltd. P. R. China), and ELISA for Herpes simplex virus (Virion-Serion R&D Co, Ltd. Germany).

2.3 Ethical compliance

The present research has received the approval of our hospital's ethics committee (approval no. 2017-042). The patients, as well as the members of their family, provided their informed consent for participation in this study, and their data were anonymized.

2.4 Statistical analysis

All data were analyzed with the help of the software SPSS version 23.0 (IBM). The results obtained from descriptive analyses are presented as absolute frequencies or rates for categorical variables, as medians (Q_{25}-to-Q_{75} values) for quantitative variables with non-parametric distribution, and as mean $\pm$ SD for quantitative variables with normal distribution. Between-group comparison of quantitative variables was performed by one-way analysis of variance or the Kruskal-Wallis test, as applicable. Comparison of categorical variables was done with the χ^2 test. Binary multivariate logistic regression analysis was used to evaluate the association of co-infection with the severity of MPP and the hospitalization period. Statistical significance was assumed at P values below 0.05.

3. Results

3.1 Demographic data

Between January 2014 and December 2017, 205 children with severe MPP were admitted to our hospital, but 10 were excluded because of incomplete clinical data. Further, in the 11 other cases that were excluded, the reason for exclusion was malnutrition in 3 cases, congenital tracheal stenosis in 4 cases, methylmalonic acidemia in 2 cases, and acute lymphoblastic leukemia in 2 cases (Fig. 1). Thus, the final number of included patients was 184 (122 boys and 62 girls; male-to-female ratio, 1.97 : 1).

The patients were classified into the following three groups: single *M. pneumoniae* infection (n = 66), mixed infection with a single noxa other than *M. pneumoniae* (n = 58), and mixed infection with two or more noxae other than *M. pneumoniae* (n = 60). These three groups were matched for gender and age ($P > 0.05$; Table 1). For the analysis, the study population was segmented into two severity levels based on the PRISM III scores: the group with score > 5 (n = 48) and the group with score ≤ 5 (n = 136) [12].

Mixed infection with viral or bacterial pathogens was detected in 118 children (64.1%), and 200 noxae were detected from 184 encounters (Table 2). In 102 check-ups (55.4%), the mixed infection involved viruses, while in 29 check-ups (15.8%), the mixed infection involved bacteria. Eleven patients tested positive for three different noxae other than *M. pneumoniae*; two patients tested positive for four different noxae other than *M. pneumoniae*; one patient tested positive for five different noxae other than *M. pneumoniae*; and one patient tested positive for six different noxae other than *M. pneumoniae*. Cytomegalovirus was isolated in 36 of the 102 (35.3%) patients with viral co-detection; Epstein-Barr virus was isolated in 30 of the 102 patients (29.4%); *Chlamydia pneumoniae*, in 19 patients (18.6%); influenza B virus, in 12 patients (11.8%); and respiratory syncytial virus, in 9 patients (8.8%).

Klebsiella pneumoniae, Escherichia coli, and Staphylococcus aureus were isolated in 5 patients each of the 29 (17.3%) patients with bacterial co-infection, and Pseudomonas aeruginosa was isolated in 4 of the 29 cases (13.8%). The percentage of patients with bacterial mixed infection was not significantly different between the three groups. There was no significant difference in the co-detection rates of noxae based on patient age or gender, or based on prematurity, respiratory comorbidity, cardiovascular complications, or adverse outcomes.

3.2 Clinical data

Fever was the most frequent symptom, and the duration of fever was not significantly different between the three groups. The proportion of infants who required invasive intubation significantly rose as the mixed infection rate increased ($P < 0.05$; Table 1). Extrapulmonary complications were also common among the patients. The proportion of patients with heart failure and extrapulmonary complications that involved two or more systems significantly differed between the three groups; however, the rates of prematurity, non-invasive ventilation, pulmonary complications, and cardiovascular complications were not significantly different. With regard to laboratory examinations, increase in the number of pathogens was associated with a progressive increase in the proportion of patients with total length of stay in hospital (TLSH) > 14 days, PCIS < 80, and mixed infection of bacteria ($P < 0.05$). A higher number of pathogens was also associated with a significant increase in TLSH, D-dimer levels, PRISM III score, and the estimated mortality based on PRISM III ($P < 0.05$). However, the WBC count, Hb level, PLT count, and RDW, as well as the CRP, PCT, PA, and LDH levels were not significantly different based on the number of pathogens (Table 1).

3.3 Correlation between mixed infection and severity of severe MPP

The distribution of the PRISM III scores was skewed; therefore, the scores were transformed with the Blom formula so that their distribution was normal. For the purpose of statistical analysis, the study population was segmented into two severity levels: the severity level was considered as 0 when the PRISM III score was ≤ 5, and it was considered as 1 when the PRISM III score was > 5. Binary logistic regression analysis was used to identify the variables that showed an independent association with the PRISM III score and PCIS. We found that mixed infection with more than one pathogen (other than

M. pneumoniae) was positively associated with the PRISM III score (β = 0.760, odds ratio [OR] = 2.139, 95% confidence interval [CI] = 1.391-2.390, P = 0.001) and PCIS (β = 1.203, OR = 3.328, 95% CI = 1.723-6.731, P = 0.000).

3.4 Correlation between mixed infection and TLSH

The distribution of the TLSH data was skewed; therefore, the data were transformed with the Blom formula to ensure the distribution was normal. For the purpose of statistical analysis, the study population was segmented into two severity levels: the severity level was considered as 0 when TLSH was $\leq$ 14 days, and it was considered as 1 when TLSH was $>$ 14 days. Binary logistic regression analysis was conducted to identify variables that showed an independent association with TLSH. We found that mixed infection with more than one pathogen (other than *M. pneumoniae*) was positively correlated with TLSH (β = 0.730, OR = 2.075, 95% CI = 1.404-3.066, P = 0.000).

4. Discussion

This study is the first to investigate the outcomes of mixed infection in pediatric patients with severe MPP. These findings are highly relevant, because in recent years, the incidence of MPP has been on the increase, and it is responsible for up to 40% of pediatric CAP cases [13].

Mixed infections are common in patients with MPP. For example, Wang *et al*. reported that viral mixed infection accounted for 37.0% of MPP cases [14], and Colin *et al*. reported a $>$ 50% rate of mixed infection in MPP cases [15]. Consistent with these studies, the prevalence of mixed infection in patients with severe MPP in this study was as high as 64.1%. Bacterial mixed infections may complicate and prolong stay in the pediatric intensive care unit [16]. In a retrospective, observational study that was conducted from 2010 to 2018, it was shown that community-acquired bacterial co-infection was common in patients with influenza-associated pneumonia, but the associated risk factors have not been identified. Additionally, bacterial mixed infection was found to be a potential predictor of disease severity, and was an independent risk factor for in-hospital mortality [17]. In the present study, too, mixed infection with bacteria, especially in cases with more than one noxa apart from *M. Pneumoniae*, was associated with higher disease severity. Further, in this study, the most commonly reported viral co-infection in children with severe MPP were cytomegalovirus and Epstein-Barr virus infection. CMV/EB reactivation may be correlated with poor prognosis [18]. But in present retrospective study, we were unable to confirm whether the children got these viruses before or not. Importantly, the findings so far indicate that interaction between multiple organisms could have adverse effects, including virus-induced airway damage, promotion of bacterial adherence, decrease in mucociliary clearance, and eventually, impairment of the immune system [19].

Steensels *et al*. reported that the presence of another pathogen was strongly associated with a higher need for supplemental oxygen [20]. A retrospective study of 2219 children hospitalized with CAP demonstrated that when compared to children who had only viral infection, children who had virus-bacterium co-infection had a higher incidence of leukocytosis, consolidation on their chest radiographs, parapneumonic effusion, admission to the intensive care unit, mechanical ventilation, and prolonged hospitalization. In contrast, virus-virus mixed infections usually have similar features to single-virus infections, except for the need for oxygen supplementation [21]. Similarly, another retrospective, single-center, cohort study of 477 children with respiratory failure demonstrated that multiviral infections were associated with longer PICU stay and prolonged mechanical ventilation [22]. In agreement with these published studies, the findings of the present study cohort also demonstrate that an increase in the number of pathogens was associated with an increase in the need for invasive mechanical ventilation and an increase in TLSH.

MPP is usually a benign self-limiting disease. However, it may sometimes cause various extrapulmonary complications and, thereby, affect the ultimate disease outcome [23]. In the present cohort, a higher number of mixed pathogens in children with severe MPP was found to be associated with an increased incidence of heart failure and extrapulmonary complications involving two or more systems. Based on these findings, it is possible that MPP precedes and intensifies subsequent infections with various respiratory viruses and bacteria, as has been previously speculated [15, 24].

Some studies have investigated the association of mixed infection with the outcome of pneumonia. For example, Loubet *et al*. reported that virus-bacteria co-infection was associated with a higher mortality rate than bacteria-only and virus-only infection (62% vs. 40% and 35%) in adults with hospital-acquired pneumonia [25]. Further, a retrospective study of 1503 adults with viral pneumonia demonstrated that viral-bacterial mixed infection was an independent predictor of mortality [26]. Another retrospective study in children hospitalized with influenza-related lower respiratory tract infection from 2008 to 2018 also reported that secondary bacterial infection was an independent risk factor for mortality [27]. Rehder *et al*. reported that children who have infection with multiple respiratory viruses may have a higher risk of moderate or severe illness and mortality, and the risk might be more pronounced in previously healthy children [28]. In the present study, binary logistic regression analysis showed that the presence of mixed infections with multiple pathogens was positively associated with TLSH and the PRISM III score and PCIS. PRISM III score, a third-generation physiology-based prediction model for mortality [29]. PCIS is widely used to evaluate the severity of illness for critically ill pediatric patients in P. R. China. Score of less than 80 means severe illness and 70 means very severe [30]. Thus, severe MPP along with mixed infection with multiple pathogens in children might be associated with an increased risk of severe illness and mortality.

The limitations of this study are its retrospective design and the lack of randomization. Nonetheless, the findings are important as a basis for further research in the area, as they establish the risk of mixed infection with multiple pathogens in MPP.

5. Conclusions

This present study demonstrated that viral and bacterial mixed infections are relatively common in severe MPP, with cytomegalovirus and Epstein-Barr virus being the most common cause of mixed infection. Importantly, mixed infection with multiple pathogens was found to be associated with increased length of hospital stay and higher disease severity, which may be indicative of higher mortality risk.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study has received the approval of the Ethics Committee of our institution (approval no. 042 (2017)). Clinical Trial Registration Code: ChiCTR1900023117.

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CONFLICT OF INTERESTS

All authors declare that there are no conflicts of interest.

AVAILABILITY OF DATA

The data sets used during the present study are available from the corresponding author upon reasonable request.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.signavitae.com/mre-signavitae/article/132677288776635187 2/attachment/SV2020091601_Supplementary%20material.docx.

REFERENCES

[1] Søndergaard MJ, Friis MB, Hansen DS, Jørgensen IM. Clinical manifestations in infants and children with Mycoplasma pneumoniae infection. PLoS One. 2018; 13: e0195288.

[2] Jiang W, Wu M, Zhou J, Wang Y, Hao C, Ji W, *et al*. Etiologic spectrum and occurrence of coinfections in children hospitalized with community-acquired pneumonia. BMC Infectious Diseases. 2017; 17: 787.

[3] Oumei H, Xuefeng W, Jianping L, Kunling S, Rong M, Zhenze C, *et al*. Etiology of community-acquired pneumoniain 1500 hospitalized children. Journal. Journal of Medical Virology. 2018; 90: 421-428.

[4] Pessoa E, Bárbara C, Viegas L, Costa A, Rosa M, Nogueira P. Factors associated with in-hospital mortality from community-acquired pneumonia in Portugal: 2000-2014. BMC Pulmonary Medicine. 2020; 20: 18.

[5] Song Q, Xu BP, Shen KL. Bacterial co-infection in hospitalized children with mycoplasma pneumoniae pneumonia. Indian Journal of Pediatrics. 2016; 53: 879-882.

[6] Han MS, Yun KW, Lee HJ, Park JY, Rhie K, Lee JK, *et al*. Contribution of co-detected respiratory viruses and patient age to the clinical manifestations of mycoplasma pneumoniae pneumonia in children. Pediatric Infectious Disease. 2018; 37: 531-536.

[7] Li Y, Pattan V, Syed B, Islam M, Yousif A. Splenic infarction caused by a rare coinfection of Epstein-Barr virus, cytomegalovirus, and Mycoplasma pneumoniae. Pediatric Emergency Care. 2014; 30: 636-637.

[8] Cawcutt KA, Kalil AC. Viral and bacterial co-infection in pneumonia: do we know enough to improve clinical care? Critical Care. 2017; 21: 19.

[9] Chiu CY, Chen CJ, Wong KS, Tsai MH, Chiu CH, Huang YC. Impact of bacterial and viral coinfection on mycoplasmal pneumonia in childhood community-acquired pneumonia. Journal of Microbiology, Immunology and Infection. 2015; 48: 51-56.

[10] Zhang X, Chen Z, Gu W, Shen N, Tao Y, Zhao R, *et al*. Viral and bacterial co-infection in hospitalised children with refractory Mycoplasma pneumoniae pneumonia. Epidemiology and Infection. 2018; 146: 1384-1388.

[11] Wang C, Song C, Wang S, Liu G. Prealbumin may predict clinical outcomes in children with severe mycoplasma pneumoniae pneumonia. Iranian Journal of Pediatrics. 2020; 30: e97680.

[12] Hsu BS, Lakhani S, Brazelton TB 3rd. Relationship between severity of illness and length of stay on costs incurred during a pediatriccriticalcare hospitalization. South Dakota Medicine. 2015; 68: 339, 341-344.

[13] Wang L, Feng Z, Shuai J, Liu J, Li G. Risk factors of 90-day rehospitalization following discharge of pediatric patients hospitalized with mycoplasma Pneumoniae pneumonia. BMC Infectious Diseases. 2019; 19: 966.

[14] Wang L, Feng Z , Zhao M , Yang S, Yan X, Guo W, *et al*. A comparison study between GeXP-based multiplex-PCR and serology assay for Mycoplasma pneumoniae detection in children with community acquired pneumonia. BMC Infectious Diseases. 2017; 17: 518.

[15] Colin AA, Yousef S, Forno E, Korppi M. Treatment of mycoplasma pneumoniae in pediatric lower respiratory infection. Pediatrics. 2014; 133: 1124-1125.

[16] Wiegers HMG, van Nijen L, van Woensel JBM, Bem RA, de Jong MD, Calis JCJ. Bacterial co-infection of the respiratory tract in ventilated children with bronchiolitis; a retrospective cohort study. BMC Infectious Diseases. 2019; 19: 938.

[17] Teng F, Liu X, Guo SB, Li Z, Ji WQ, Zhang F, *et al*. Community-acquired bacterial co-infection predicts severity and mortality in influenza-associated pneumonia admitted patients. Journal of Infection and Chemotherapy. 2019; 25: 129-136.

[18] Cantan B, Luyt CE, Martin-Loeches I. Influenza infections and emergent viral infections in intensive care unit. Seminars in Respiratory and Critical Care Medicine. 2019; 40: 488-497.

[19] Cawcutt K, Kalil AC. Pneumonia with bacterial and viral coinfection. Current Opinion in Critical Care. 2017; 23: 385-390.

[20] Steensels D, Reynders M, Descheemaeker P , Curran MD, Hites M, Etienne I, Montesinos I. Epidemiology and clinical impact of viral, atypical, and fungal respiratory pathogens in symptomatic immuno-compromised patients: a two-center study using a multi-parameter customized respiratory Taqman® array card. European Journal of Clinical Microbiology and Infectious Diseases. 2019; 38: 1507-1514.

[21] Nolan VG, Arnold SR, Bramley AM, Ampofo K, Williams DJ, Grijalva CG, *et al.* Etiology and impact of coinfections in children hospitalized with community-acquired pneumonia. Journal of the Infectious Diseases. 2018; 218: 179-188.

[22] Chauhan JC, Slamon NB. The impact of multiple viral respiratory infections on outcomes for critically Ill children. Pediatric Critical Care Medicine. 2017; 18: e333-e338.

[23] D'Alonzo R, Mencaroni E, Di Genova L, Laino D, Principi N,Esposito S. Pathogenesis and treatment of neurologic diseases associated with mycoplasma pneumoniaeinfection. Frontiers in Microbiology. 2018; 9: 2751.

[24] Kinnula H, Mappes J, Sundberg LR. Coinfection outcome in an opportunistic pathogen depends on the inter-strain interactions. BMC Evolutionary Biology. 2017; 17: 77.
[25] Loubet P, Voiriot G, Houhou-Fidouh N , Neuville M, Bouadma L, Lescure FX, *et al*. Impact of respiratory viruses in hospital-acquired pneumonia in the intensive care unit: A single-center retrospective study. Journal of Clinical Virology. 2017; 91: 52-57.
[26] Kim YJ, Lee ES , Lee YS. High mortality from viral pneumonia in patients with cancer. Infectious Diseases(London). 2019; 51: 502-509.
[27] Eşki A, Öztürk GK, Gülen F, Çiçek C, Demir E. Risk factors for influenza virus related severe lower respiratory tract infection in children. Pediatric Infectious Disease Journal. 2019; 38: 1090-1095.
[28] Rehder KJ, Wilson EA, Zimmerman KO, Cunningham CK, Turner DA. Detection of multiple respiratory viruses associated with mortality and severity of illness in children. Pediatric Critical Care Medicine. 2015; 16: e201-6.
[29] Sayed HA, Ali AM, Elzembely MM. Can pediatric risk of mortality Score (PRISM III) be used effectively in initial evaluation and follow-up of critically Ill cancer patients admitted to pediatric oncology intensive care unit(POICU)? A prospective study, in a tertiary cancer center in Egypt. Journal Pediatric Hematology and Oncology. 2018; 40: 382-386.
[30] Ho K, Wang X, Chen L.Reasons for parental withdrawal of care in a pediatric intensive care unit in P. R. China. PLoS One. 2018; 13: e0199419.

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