Early Discrimination of Refractory Mycoplasma Pneumoniae Pneumonia in Children: A Multicenter Prospective Study in Zhejiang, China

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Abstract

Objective: To explore potential predictors of RMPP in early stage.

Study design: The prospective study, multicenter study was conducted in Zhejiang, China from May 1st, 2019 to January 31st, 2020. Children aged 29 days to 14 years old, with fever time during 48 to 120 hours were included. A total of 1428 children completed the study. A questionnaire was designed to collect patients' information. Pharyngeal swab samples were collected. M. pneumoniae DNA in pharyngeal swab specimens were detected. Whether the patients develop to RMPP were assessed. Logistic regression analyses were used to examine associations between clinical data and RMPP.

Results: The ages of the patients ranged from 34 days to 13.9 years with a median 4.3 years. The positive rate of *M. pneumoniae* -DNA was 37.4% (534/1428), and 446 cases were *Mycoplasma pneumoniae* pneumonia (MPP). In MPP patients, 55 cases were RMPP (12.3%), others were general MPP (GMPP) patients (n=391, 87.7%). Only the peak body temperature before the first visit and LDH level in RMPP patients were higher than that in GMPP [39.6 (39.1-40.0) °C vs. 39.2 (38.9-39.7) °C, p=0.003, and 332.5 (278.8-392.1) U/L vs. 310.5 (259.0-358.8) U/L, p=0.024]. Logistic regression also only included the above two parameters in the prediction probability. The area under ROC curve of the prediction probability π of RMPP was 0.682 (95% CI, 0.593-0.771), P<0.01. The cut-off value was 0.12. Sensitivity and specificity of the prediction probability π in cut-off value was 0.64 and 0.70, respectively.

Conclusions: AND RELEVANCE A prediction probability, calculating from the peak body temperature before the first visit and LDH level for early identifying RMPP from other MPP within 2-5 days of fever duration, with a cut-off value of 0.12 may be helpful in clinical practice.

Introduction

Mycoplasma pneumoniae (*M. pneumoniae*) is one of the most important pathogens causing community-acquired pneumonia (CAP) in children. In most studies, 10-30% of cases of CAP are due to *M. pneumoniae* [1]. Although *Mycoplasma pneumoniae* pneumonia (MPP) is usually a self-limited disease, some cases are severe and life-threatening [2-4]. It may also cause a variety of intrapulmonary and extrapulmonary complications such as pleural effusion, atelectasis, pulmonary consolidation, pleural thickening, encephalitis, arthritis, pericarditis, and anemia, and even lead to sequelae and disability, such as bronchiectasis, bronchiolitis obliterans, interstitial pulmonary fibrosis, and paralysis [5,6].

Despite appropriate antibiotic, some patients still progress to a stage called refractory *Mycoplasma pneumoniae* pneumonia (RMPP), which was defined as MPP showing clinical and radiological deterioration despite macrolide antibiotic therapy for 7 d or longer. Corticosteroids have been used with satisfactory therapeutic effect for children with RMPP [7,8], which induce excessive immunological inflammation in the body. Due to a high percentage of macrolide-resistant *M. pneumoniae*, tetracyclines and fluoroquinolones were also used in RMPP children [9,10]. However, all of the mentioned medicines are

with potential risk of causing side effects in children, especially in small children. Thus, to lessen the suffering of patients, shorten the disease duration, reduce unnecessary medication use, identifying potential RMPP patients in the early stage is very important. To data some studies have been reported that serum lactate dehydrogenase (LDH), interleukin (IL)-6, IL-10, IL-18, IFN-γ and C-reactive protein (CRP) could be used as parameters to aid in early recognition of RMPP [3,11-13]. However, large scale prospective studies are rare. Herein we conducted a multi-center, prospective study to explore potential predictors of RMPP.

Methods

2.1 Patients

Patients were enrolled from 13 hospitals in Zhejiang, China from May 1st, 2019 to January 31st, 2020. Inclusion criteria were: (1) Aged 29 days to 14 years old; (2) Body temperature: core temperature > 37.7 C; (3) fever time > 48 hours. Exclusion criteria were: (1) Fever caused by obvious non-respiratory infection; (2) Congenital immune deficiency, severe respiratory and circulatory diseases, chronic lung diseases, kidney or liver diseases, cardiovascular diseases and connective tissue diseases; (3) Long-term use of systemic hormones or immunosuppressants; (4) Fever time is more than 120 hours; (5) Respiratory tract infection and use of macrolide antibiotics within two months; (6) Complicated with chickenpox, hand, foot and mouth disease or herpes angina; (7) Allergic to azithromycin, minocycline and methylprednisolone; (8) Parents or children are unwilling to participate in the research. Exit criteria: (1) Those who are allergic to azithromycin or minocycline or methylprednisolone; (2) Parents or children request to withdraw from the study; (3) Incomplete medical records.

Patients who meet the criteria within the study time were all enrolled to minimize selection bias in selecting patients.

The study was approved by the Ethics Committee of Children's Hospital, Zhejiang University School of Medicine (2019-IRB-058) and was registered at Chinese Clinical Trial Registry (Registration number ChiCTR1900023908). All methods were performed in accordance with the relevant guidelines and regulations. Agreement and signed written consents were received from their guardians and themselves if they were older than 8 years.

2.2 Data collection

A questionnaire was designed to collect patients' information, including age, gender, clinical signs and symptoms, past history, allergy history (eczema history, food allergy history, drug allergy history, allergic rhinitis history) and family history, family members (number of people, accommodation conditions). Results of laboratory test, including complete blood count, CRP, LDH, procalcitonin (PCT), chest radiograph results, and management were also recorded.

Pharyngeal swab samples were collected. *M. pneumoniae* DNA in pharyngeal swab specimens were detected.

Children with pneumonia may present with signs and symptoms of fever, cough, wheeze, tachypnea, breathlessness or difficulty in breathing, abnormal lung auscultation and/or an abnormal manifestation on chest radiograph [14]. MPP patients were pneumonia patients with *M. pneumoniae* infection, which was diagnosed by a positive result of PCR.

Patients with MPP were followed up for one more week (mainly in outpatient clinic, some by telephone) to observe whether the patients deteriorated into RMPP. Diagnostic criteria of RMPP were MPP patients, who were treated with macrolides for 7 days or more, had aggravation of clinical signs, continued fever and aggravation of pulmonary imaging. Other MPP patients were referred to as general MPP (GMPP) patients.

2.3 Qualitative detection of *M. pneumoniae*

M. pneumoniae DNA in pharyngeal swab specimens were detected by fluorescence quantitative real-time PCR, which was performed according to "*M. pneumoniae* nucleic acid test kit (fluorescent probe-based real-time PCR assay)" (Jangsu Mole Bioscience Co., Ltd, China). The kit was approved by State Food and Drug Administration of China as clinical diagnostic kit.

Statistical analysis:

Normally distributed quantitative data were reported as mean ± standard deviation and compared by Student's t test. Skew distributed quantitative data were reported as median (25^{th}-75^{th} interquartile range) and compared by Mann-Whitney U test. Chi-square test or Fisher's exact test was applied for qualitative data comparison. Logistic regression analyses were used to examine associations between clinical data and RMPP. Receiver operating characteristic (ROC) curves were used to analyse the power of the markers for prediction of RMPP. Variables were considered statistically significant at a *P* values of less than 0.05 by using two-sided tests. Missing values were filled with a constant "null". Data were analyzed by SPSS 19.0 (SPSS Inc., USA).

Results

3.1 Patient characteristics

The children were enrolled from 13 hospitals (Table 1). Hospitals are at different levels. There are five county level hospitals, seven city level hospitals and one provincial level hospital.

There were 1560 patients enrolled. There were 132 children were excluded because of incomplete results or information or other reasons. A total of 1428 children completed the study. The ages of the patients ranged from 34 days to 13.9 years with a median 4.3 years (Fig 1). The number of boys was 801 (56.1%).

Table 1 Patients from different hospitals

Hospital	Number of patients
Ningbo Women and Children's Hospital	181
Sanmen People's Hospital	174
Shaoxing Second Hospital	171
Changxing Maternity and Child Health Care Hospital	165
Ningbo Medical Center Lihuili Hospital	164
Taizhou Hospital of Zhejiang Province	144
The Children’s Hospital, Zhejiang University School of Medicine	127
Zhoushan Women and Children Hospital	83
Cixi Maternity and Child Health Care Hospital	57
Quzhou Maternal and Child Health Care Hospital	47
Huzhou Central Hospital	45
The Second Affiliated Hospital of Jiaxing University	41
Shengsi People's Hospital	29

3.2 Comparison of *M. pneumoniae* -DNA positive and negative patients

The positive rate of *M. pneumoniae* -DNA was 37.4% (534/1428). Positive rate of *M. pneumoniae* were different in different hospitals (Fig 2). Gender proportion had no significant difference between the groups (boys 55.6% vs. 56.4%, χ^2=0.078，p=0.780).

The number of *M. pneumoniae* -DNA positive and negative patients was 534 (37.4%) and 894 (62.6%). The median ages of *M. pneumoniae* -DNA positive and negative patients were 5.2 and 3.8 years, respectively, with significant difference between the two groups (p<0.001).

Despite we enrolled patients with fever durations between 2 and 5 days, the fever and cough duration on the first visit had significant difference between *M. pneumoniae* -DNA positive and negative patients (both p<0.001, Table 2). The proportion of other symptoms, including wheezing, tachypnea, pharyngalgia, chest pain, headache, hoarseness, convulsion, abdominal pain and rash had no significant differences between *M. pneumoniae* -DNA positive and negative patients (all p >0.05).

In laboratory test, LDH, CRP and neutrophil percentage in *M. pneumoniae* -DNA positive patients were higher than in *M. pneumoniae* -DNA negative ones. WBC and lymphocyte percentage were lower in *M. pneumoniae* -DNA positive patients than in *M. pneumoniae* -DNA negative ones. Other parameters were also listed in Table 2.

Table 2 Comparison of laboratory test between M. pneumoniae -DNA positive and negative patients

	M. pneumoniae - DNA positive (n=534)	*M. pneumoniae* - DNA negative (n=894)	p
Gender (boy/girl, n)	297/237	504/390	0.780
Age [median (25th-75th interquartile range), years old, 1428 valid data]	5.2 (3.3-7.0)	3.8 (2.2-5.7)	0.000
Symptoms			
Fever [median (25th-75th interquartile range), day, 1428 valid data]	4 (3-4)	3 (2-4)	0.000
Cough [median (25th-75th interquartile range), day, 1428 valid data]	3 (2-5)	2 (0-4)	0.000
Wheeze [median (25th-75th interquartile range), day, 1428 valid data]	0 (0-0)	0 (0-0)	0.220
Tachypnea [n (%), 1428 valid data]	2 (0.4%)	4 (0.4%)	0.598
Pharyngalgia [n (%), 1428 valid data]	1 (0.2%)	6 (0.7%)	0.195
Chest pain [n (%), 1428 valid data]	0 (0%)	1 (0.1%)	0.626
Headache [n (%), 1428 valid data]	0 (0%)	2 (0.2%)	0.392
Hoarseness [n (%), 1428 valid data]	3 (0.6%)	8 (0.9%)	0.360
Convulsion [n (%), 1428 valid data]	0 (0%)	2 (0.2%)	0.392
Abdominal pain [n (%), 1428 valid data]	0 (0%)	1 (0.1%)	0.626
Rash [n (%), 1428 valid data]	1 (0.2%)	6 (0.7%)	0.195
The peak body temperature [median (25th-75th interquartile range),°C, 1303 valid data]	39.3 (39.0-39.8)	39.4 (39.0-39.9)	0.177
Laboratory values			
PCT [median (25th-75th interquartile range), ng/ml, 903 valid data]	0.1 (0-0.2)	0.1 (0-0.2)	0.225
LDH [median (25th-75th interquartile range), U/L, 912 valid data]	312.0 (262.5-361.0)	291.0 (251.9-345.0)	0.002
WBC [median (25th-75th interquartile range), $\times 10^9$/L, 1376 valid data]	7.2 (6.0-8.8)	7.9 (5.6-11.3)	0.000
L [X±SD, %, 1371 valid data]	32.2±11.3	34.9±21.1	0.003
CRP [median (25th-75th interquartile range), mg/L, 1370 valid data]	12.0 (5.9-21.4)	8.8 (2.6-23.6)	0.000
N [X±SD, %, 1376 valid data]	57.4±13.5	55.0±18.7	0.006

3.3 Comparison of RMPP and GMPP patients

In the 534 *M. pneumoniae* -DNA positive patients, 446 cases were MPP. In MPP patients, 55 cases were RMPP (12.3%), others were GMPP patients (n=391, 87.7%).

The proportion of boys in RMPP and GMPP were 50.9% (28/55) and 57.0% (223/391), the gender ratio had no significant statistical difference between RMPP and GMPP (χ^2=0.735, p=0.391). The median age of RMPP and GMPP were 5.0 and 5.2 years, respectively, with no significant statistical difference (p=0.890).

Fever duration, cough duration, and the proportion of other symptoms, including wheezing, tachypnea, pharyngalgia, chest pain, headache, hoarseness, convulsion, abdominal pain and rash had no significant differences between RMPP and GMPP (all p >0.05) (Table 3). The peak body temperature before the first visit in RMPP patients were higher than that in GMPP [39.6 (39.1-40.0) °C vs. 39.2 (38.9-39.7) °C, p=0.003].

In laboratory tests, LDH were higher in RMPP patients than in GMPP ones [332.5 (278.8-392.1) U/L vs. 310.5 (259.0-358.8) U/L, p=0.024]. PCT, CRP, WBC, neutrophil percentage, lymphocyte percentage had no significant differences between RMPP and GMPP (all p >0.05) (Table 3).

In radiological features, the proportions of thickening of lung marking bronchopneumonia, consolidation, atelectasis, plural effusion, emphysema and thickening of hilar shadow had no significant differences between RMPP and GMPP (all p >0.05) (Table 3).

In past history and family history, we investigated exposure to other fever patients within one week, patients', parents' and grandparents' food and medicine allergy, patients', parents' and grandparents' asthma, eczema, hay fever history, number of people living together expect the patient, sibling numbers, gravidity and parity number, preterm born, maternal breast feeding time, multiple birth. All of them had no significant differences between RMPP and GMPP (all p >0.05) (Table 3).

In treatment before the first visit, macrolides were applied to 27 (49.1%) and 163 (41.7%) patients in RMPP and GMPP, with no significant differences between them (χ^2=1.081, p=0.299).

Table 3 Characteristics of RMPP and GMPP patients

	RMPP (n=55)	GMPP (n=391)	p
Gender (boy/girl, n)	28/27	223/168	0.391
Age [median (25th-75th interquartile range), years old, 446 valid data]	5.0 (3.0-8.0)	5.2 (3.3-6.8)	0.890
Macrolides-resistant mutation [n (%), 446 valid data]	42 (76.4%)	368 (94.1%)	0.939
Symptoms			
Fever [median (25th-75th interquartile range), day, 446 valid data]	4 (3-5)	4 (3-4)	0.517
Cough [median (25th-75th interquartile range), day, 446 valid data]	3 (2-5)	4 (2-5)	0.397
Wheeze [median (25th-75th interquartile range), day, 446 valid data]	0 (0-0)	0 (0-0)	0.231
Tachypnea [n (%), 446 valid data]	0 (0%)	2 (0.5%)	1.000
Pharyngalgia [n (%), 446 valid data]	0 (0%)	0 (0%)	/
Chest pain [n (%), 446 valid data]	0 (0%)	0 (0%)	/
Headache [n (%), 446 valid data]	0 (0%)	0 (0%)	/
Hoarseness [n (%), 446 valid data]	0 (0%)	1 (0.3%)	1.000
Convulsion [n (%)], 446 valid data	0 (0%)	0 (0%)	/
Abdominal pain [n (%), 446 valid data]	0 (0%)	0 (0%)	/
Rash [n (%), 446 valid data]	1 (1.8%)	0 (0%)	0.123
The peak body temperature [median (25th-75th interquartile range),°C , 380 valid data]	39.6 (39.1-40.0)	39.2 (38.9-39.7)	0.003
Laboratory values			
PCT [median (25th-75th interquartile range), ng/ml, 342 valid data]	0.11 (0.05-0.24)	0.08 (0.04-0.14)	0.148
LDH [median (25th-75th interquartile range), U/L, 348 valid data]	332.5 (278.8-392.1)	310.5 (259.0-358.8)	0.024
WBC [median (25th-75th interquartile range), ×109/L, 439 valid data]	7.0 (5.9-8.4)	7.2 (5.9-8.8)	0.823
L [X±SD, %, 439 valid data]	30.8±11.7	33.0±11.5	0.181
CRP [median (25th-75th interquartile range), mg/L, 438 valid data]	15.3 (6.8-29.0)	11.5 (5.7-19.8)	0.062

N [X±SD, %, 439 valid data]	59.3±12.3	56.8±13.2	0.195
Radiological features [n (%)]			
Thickening of lung marking (393 valid data)	2 (4.3%)	19 (5.5%)	1.000
Bronchopneumonia (446 valid data)	22 (40.0%)	152 (38.9%)	0.759
Consolidation (446 valid data)	28 (50.9%)	169 (43.2%)	0.436
Atelectasis (446 valid data)	1 (1.8%)	4 (1.0%)	0.709
Plural effusion (446 valid data)	3 (5.5%)	6 (1.5%)	0.177
Emphysema (446 valid data)	0 (0%)	1 (0.3%)	0.746
Thickening of hilar shadow (446 valid data)	0 (0%)	3 (0.8%)	0.681
Personal and family history			
Exposure to other fever patients within one week [n (%), 446 valid data]	3 (6.1%)	37 (12.5%)	0.237
Food and medicine allergy [n (%), 446 valid data]	2 (3.9%)	23 (6.4%)	0.755
Asthma, eczema, hay fever history [n (%), 408 valid data]	3 (5.9%)	36 (10.1%)	0.450
Parents' and grandparents' food and medicine allergy [n (%), 409 valid data]	0 (0%)	1 (0.3%)	1.000
Parents' and grandparents' asthma, eczema, hay fever history [n (%), 409 valid data]	1 (2.0%)	7 (2.0%)	1.000
Number of people living together expect the patient [median (25th-75th interquartile range), n, 409 valid data]	3 (2-3)	3 (2-3)	0.572
Sibling numbers [median (25th-75th interquartile range), n, 357 valid data]	1 (0-1)	0 (0-1)	0.254
Gravidity order [median (25th-75th interquartile range), n, 406 valid data]	1 (1-2)	1 (1-2)	0.017
Birth order [median (25th-75th interquartile range), n, 406 valid data]	1 (1-2)	1 (1-2)	0.342
Preterm born [n (%), 394 valid data]	0 (0%)	6 (1.7%)	1.000
Maternal breast feeding [median (25th-75th interquartile range), month, 400 valid data]	7 (6-12)	9 (6-12)	0.924
Multiple birth [n (%), 408 valid data]	1 (2.0%)	5 (1.4%)	0.538

Logistic regression results was showed in Table 4. Only the peak body temperature and LDH were included. The prediction probability π= exp [-29.7+0.667×Peak body temperature (°C) + 0.004×LDH (U/L)]/{1+exp [-29.7+0.667×Peak body temperature (°C) + 0.004×LDH (U/L)]} (Fig 3). The forecast

consensus percentage was 88.8%. The area under ROC curve (Fig 4) of the prediction probability π of RMPP was 0.682 (95% CI, 0.593-0.771), P<0.01. The cut-off value was 0.12. Sensitivity and specificity of the prediction probability π in cut-off value was 0.64 and 0.70, respectively.

Table 4. Logistic regression analysis for related factors predicting the RMPP

Variable	B	S.E.	Wald	P value	Exp (B)	OR	95%CI	
							Lower	Upper
Peak body temperature (°C)	.667	.284	5.522	.019	1.949	1.949	1.117	3.401
LDH (U/L)	.004	.002	6.547	.011	1.004	1.004	1.001	1.007
Constant	-29.700	11.178	7.060	.008	.000			

3.4 Clinical course of RMPP

Despite 42 (76.4%) RMPP patients were found macrolides-resistant mutation positive, macrolide was applied to all of them. Doxycycline was used in 2 (3.6%) patients. Systemic corticosteroid was used in 52 (94.5%) RMPP patients. The duration of systemic corticosteroid was 5 (3-6) days. Intravenous immunoglobin was used in 2 (3.6%) patients. One patient underwent bronchoscopy because of persistent atelectasis. The total fever duration was 8 (7-9) days.

Discussion

Although CAP due to *M. pneumoniae* is usually benign, in some patients, it proceeds to RMPP, even causes a life-threatening condition [15]. Because CAP due to *S. pneumoniae* is more common than CAP due to *M. pneumoniae*, physicians prescribe more beta-lactams, which is not suitable for MPP, than macrolides. However, despite the appropriate use of macrolide antibiotics and/or corticosteroids therapy, there are still chances for aggravation of MP infection. In recent years, an increasing number of patients with RMPP are being reported [16-20]. As early intervention is important in outcome improving [6], it is very important to early recognize RMPP. The first step is to recognize MPP from other CAP. We applied fluorescence quantitative real-time PCR for rapid detection of *M. pneumoniae* infection. The second step is to recognize RMPP from other MPP.

This multi-center, prospective study investigated 1428 cases of fever patients prospectively. Patients were from 13 hospitals, which scattered in most area of Zhejiang province. The hospitals are from county to city to provincial levels to represent hospitals in different levels.

M. pneumoniae -DNA patients counted for 37.4% (n=534). In the comparison between *M. pneumoniae* -DNA positive and negative patients, we found age, fever duration, cough duration, LDH, CRP and neutrophil percentage in *M. pneumoniae* -DNA positive patients were higher than in *M. pneumoniae* -DNA

negative ones. WBC and lymphocyte percentage were lower in *M. pneumoniae* -DNA positive patients than in *M. pneumoniae* -DNA negative ones. These parameters are helpful in differentiate *M. pneumoniae* -infection from other infections.

In the *M. pneumoniae* -DNA positive patients, 446 cases were MPP. In MPP patients, we identified 55 RMPP (12.3%). The proportion of RMPP in MPP is similar to 12.9% in a single-center prospective study [16], which was conducted in an adjacent province of Zhejiang. In the comparison between RMPP and GMPP, the peak body temperature and LDH were both higher in RMPP patients than those in GMPP patients (both $p<0.05$). We also obtained a prediction probability to predict RMPP in MPP who were in early stage (2 to 5 days). Despite we calculate all the parameters, including signs, symptoms, laboratory tests, and radiological features, personal and family history, the mathematical formula only includes the peak body temperature and LDH, which were both easily accessible in outpatient clinic setting. The area under ROC curve (Fig 4) of the prediction probability π of RMPP was 0.682 (95% CI, 0.593-0.771), $P<0.01$. The cut-off value was 0.12. Patients of MPP with high prediction probability are at high risk of developing to RMPP.

Scholars have been dedicated to find predictors or markers for RMPP. LDH, CRP, IL-6, TNF-α, aspartate aminotransferase, alanine aminotransferase, IL-18, long duration of fever and presence of mucus plugs were also reported as parameters to identify RMPP patients with long time of radiographic clearance [13,16,21]. High LDH, CRP, IL-10, IFN-γwere biomarkers for predicting corticosteroid-resistant RMPP [18,19]. In other literatures, some mathematical formulas also have been reported to predict RMPP. A stepwise logistic regression analysis in a retrospective study predicting the RMPP included sex, hydroxybutyrate dehydrogenase, CRP, febrile day before admission, and mucus plug under bronchoscopy [22]. In Zhang's study [12], a logistic regression analysis included CRP ≥16.5mg/L, LDH ≥417 IU/L and IL-6 ≥14.75pg/ml to predict RMPP. LDH is a ubiquitous enzyme, which catalyzes the oxidative conversion of the substrate pyruvate to lactate and elevated in many inflammatory processes. It can be found in all tissues, but only within the cytoplasm rather than secretion. Thus, serum LDH reflects disruptions of cell membranes or cell damage [23]. It is a suggested biomarker for the use of steroid therapy [4]. The cut-off level of LDH was 379 IU/L to 417 IU/L [5,12,13]. The underlying mechanisms of RMPP are still unknown. LDH and other immune reactions may indicate lung injury. It is reasonable to recommend for early use of immune modulators. Thus, early corticosteroids or even bronchoalveolar lavage therapies with proper indications may be applied early in the hope of preventing processing to RMPP or at least shortening the fever duration.

Except the peak body temperature and LDH, all the other parameters, including gender, age, macrolides-resistant mutation, symptoms, laboratory values, radiological features, personal and family history did not have statistical significant differences between RMPP and GMPP. All the other parameters had no statistical significant differences in the logistic regression analysis either. Our study enrolled patients within 2-5 days of fever duration and did not record the dynamic changes of those parameters. The laboratory values and radiological features change with disease process. It is reasonable that parameters are not sufficient enough to differentiate RMPP from GMPP in early stage.

This study has some limitations. First, the study was conducted in Zhejiang province. Although the hospitals participated cover most part of Zhejiang and are from county to provincial levels, the results are sufficient to illustrate status of Zhejiang but may not be representative for other area. Second, the study were mostly conducted in outpatient clinic without thorough investigation of pathogen detection, the co-infection of other pathogens were not considered. For the same reason, many other laboratory parameters or lung function were also not recorded, so we are not able to explore the relationship of them between RMPP and GMPP, especially for immunological parameters. In addition, the level of LDH changes with the disease process [13], we did not follow the parameter dynamically. Despite these limitations, to our knowledge, this is the first prospective multi-center prospective study that explored predictors for RMPP.

In conclusion, we conducted a prospective multi-center prospective study in Zhejiang, China. Thirteen hospitals from county level to provincial level in most part of Zhejiang province participated. In the 1428 patients completed investigated, fluorescence quantitative real-time PCR showed *M. pneumoniae* -DNA positive in 534 (37.4%) patients, among whom 446 cases were MPP. In MPP patients, 55 cases were RMPP (12.3%), others were GMPP patients (n=391, 87.7%). The peak body temperature before the first visit and LDH level in RMPP patients were higher than that in GMPP. A prediction probability, calculating from the peak body temperature before the first visit and LDH level for early identifying RMPP from other MPP within 2-5 days of fever duration, with a cut-off value of 0.12 may be helpful in clinical practice.

Abbreviations

RMPP Refractory *Mycoplasma pneumoniae* pneumonia

ROC Receiver operating characteristic

GMPP General *Mycoplasma pneumoniae* pneumonia

M. pneumoniae Mycoplasma pneumoniae

CAP Community-acquired pneumonia

MPP *Mycoplasma pneumoniae* pneumonia

LDH Lactate dehydrogenase

CRP C-reactive protein

IL Interleukin

PCT Procalcitonin

Declarations

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Author Contributions: Y Wang and Z Chen had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Supervision: Y Wang, Z Chen

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Figures

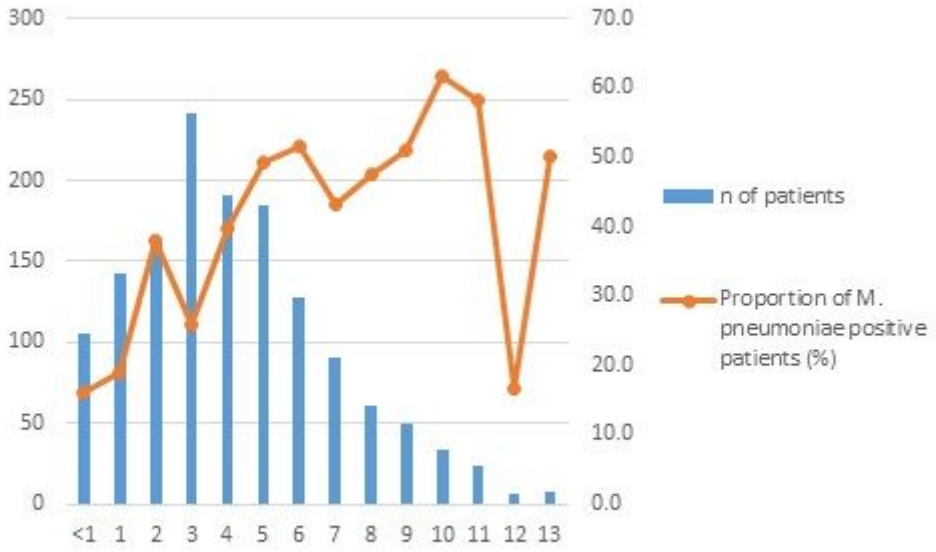

Figure 1

The age distribution of all the patients enrolled and positive rates of M. pneumoniae

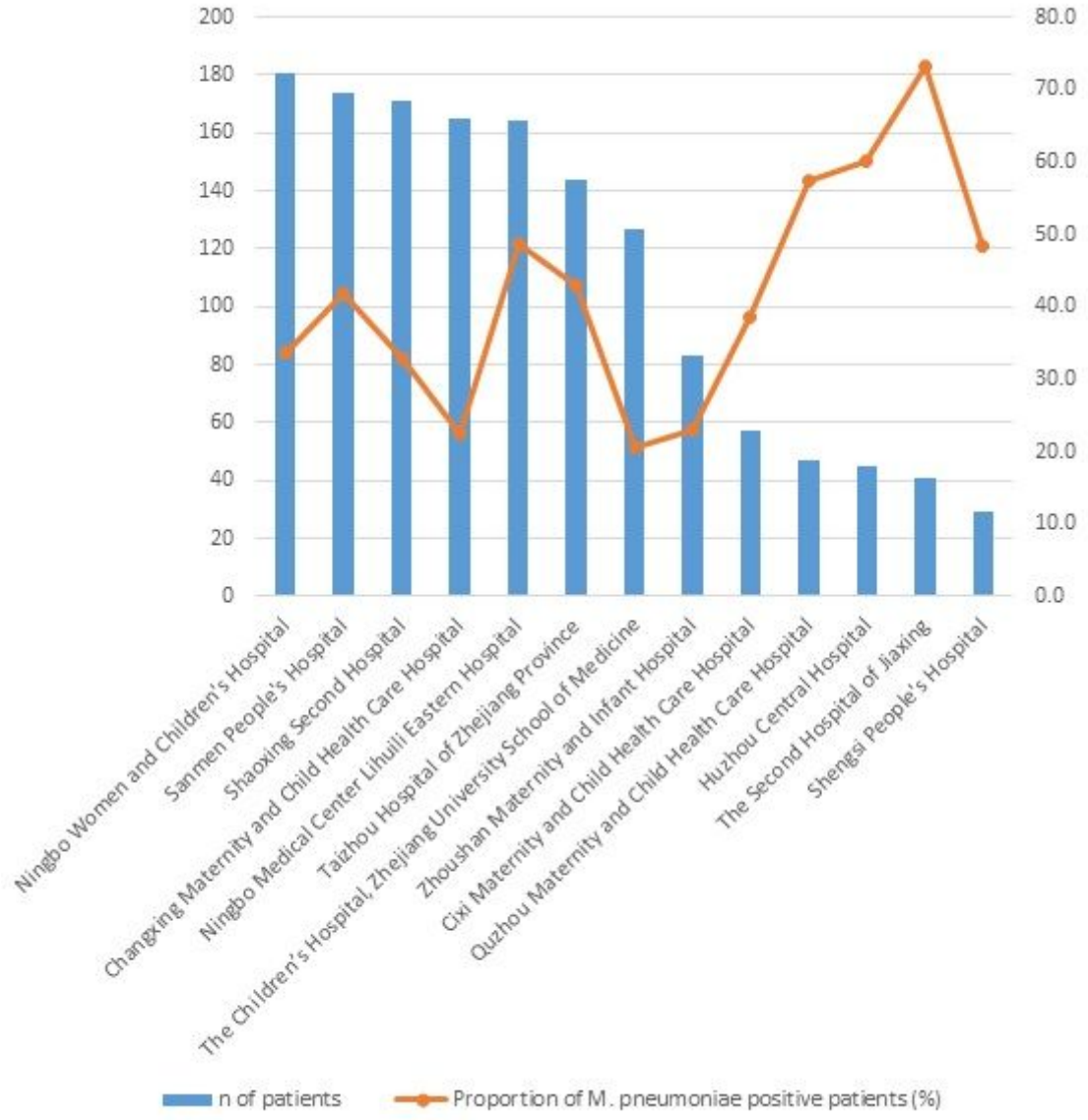

Figure 2

The hospital distribution of all the patients enrolled and positive rates of M. pneumoniae

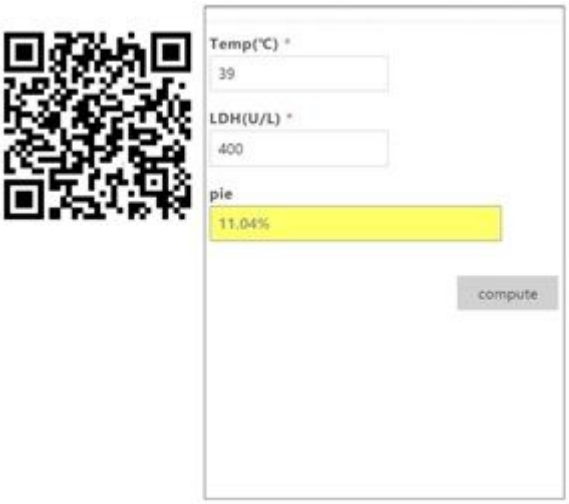

Figure 3

This quick response code provide a quick way to get a prediction probability π through scanning the code by Wechat, a China's popular messaging app, or through http://122.224.127.42:9090/interface/hzjjNew/forcast.jsp. The prediction probability is for identifying RMPP from other MPP within 2-5 days of disease onset

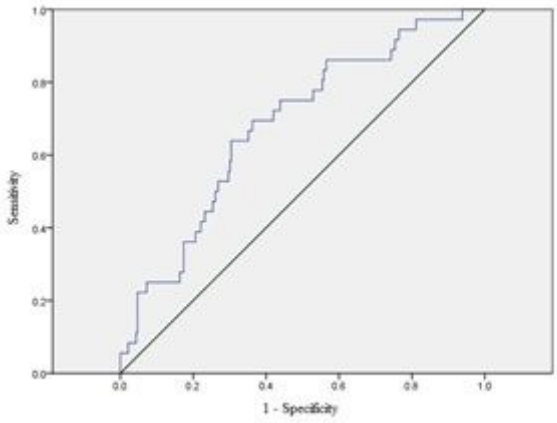

Figure 4

ROC curve of the prediction probability for identifying RMPP from other MPP within 2-5 days of disease onset.