Insights into the Therapeutic of Glucocorticoid for Refractory Mycoplasma Pneumoniae Pneumonia in Children

Zhenli Zhu
Tianjin Medical University

Tongqiang Zhang
Tianjin Agricultural University

Wei Guo
Tianjin Children's Hospital

Yaoyao Ling
Tianjin Medical University

Jiao Tian
Tianjin Medical University

Yongsheng Xu (✉ 1139350425@qq.com)
Tianjin Children's Hospital  https://orcid.org/0000-0002-5182-2819

Research article

Keywords: Refractory mycoplasma pneumoniae pneumonia, Children, Glucocorticoid

DOI: https://doi.org/10.21203/rs.3.rs-56843/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Objective To observe the efficacy and safety of different doses of glucocorticoid for refractory mycoplasma pneumoniae pneumonia in children, analyze the clinical characteristics in different groups of patients, and explore the factors related to affect illness severity for children with refractory mycoplasma pneumoniae pneumonia and guide the dosage of glucocorticoids.

Methods Retrospective analysis was performed on 279 children with refractory mycoplasma pneumoniae pneumonia hospitalized in our hospital between September 2018 and October 2019. 23 children were excluded, the remaining 256 children were divided into three subgroups: Group I was not given methylprednisolone (n=75), group II (n=115) was given methylprednisolone ≤125mg/d, and group III was given methylprednisolone >125mg/d (n=66). The clinical features, laboratory data, radiological manifestations between three subgroups of children were compared, relevant indicators with meaningful were used for ROC curve and multiple logistic regression analysis, and the optimal values of related factors were analyzed.

Results The median age and median weight of the group III were greater than the group II (P<0.05), the median age and median weight of the group I were greater than the group II (P<0.05), there was no statistical significance in median age and median weight between group III and group II (P>0.05). The group II is more serious than that of group I, and group III is more serious than that of group II, higher incidence of hypoxemia, longer fever, longer hospital stays, higher incidence of extrapulmonary complications, and more severe of radiological findings (P<0.05). The more severe presentation of disease, hormones dosage was larger, the use rate of gamma globulin was higher, the use rate of bronchoscopy was higher, and higher incidence of plastic bronchitis (P<0.05). Meanwhile, WBC, CRP, LDH, FER, D-D dimer, APTT, PLT, PCT, IL-6, ALT and the percentage of neutrophils in the three groups showed a gradual upward trend (P<0.05). In ROC curve analysis, WBC, neutrophils percentage, CRP, LDH, Fer, PCT and IL-6 can be used to distinguish RMPP with different severity and to guide the dosage of glucocorticoids. Multivariate logistic regression analysis showed that LDH 424.5IU/L, PCT 0.145ng/ml, IL-6 26.69pg/ml and lung consolidation were significant predictors for the severity of RMPP and glucocorticoids dose.

Conclusions LDH 424.5IU/L, PCT 0.145ng/ml, IL-6 26.69pg/ml and pulmonary consolidation as markers of disease severity in patients with RMPP and the dosage of glucocorticoids, which can aid in early recognition of children with severe illness, use appropriate doses of hormones, and reduce sequelae.

Introduction

Mycoplasma Pneumoniae (MP) is one of the major pathogeneses of Community Acquired Pneumonia (CAP) in children[1]. Mycoplasma Pneumoniae Pneumonia (MPP) is usually considered as a benign and self-limiting disease. However, it has been found in clinical practice that some children still progress to refractory mycoplasma pneumoniae pneumonia(RMPP) after being treated with sufficient and long-term
macrolide antibiotics in timely\[2-7\], it often leads to serious internal and external pulmonary complications, such as pulmonary necrosis and pleural effusion, which may not only difficult to treat and increase medical expenses, but also leave sequela such as bronchiectasis, atelectasis, necrotizing pneumonia and bronchiolitis obliterans\[8-12\], affecting the quality of life. More worryingly, the prevalence of RMPP is increasing due to the abuse of macrolides and the emergence of drug-resistant strains\[13,14\]. Therefore, on the basis of adequate anti-infective treatment, immunotherapy, especially the combination therapy of glucocorticoids, has attracted more and more attention\[6,15\]. It is very important for clinicians to identify RMPP of different severity as early as possible and grasp the appropriate opportunity for reasonable therapy. So, Retrospective analysis was performed on 279 children with refractory mycoplasma pneumoniae pneumonia (RMPP) hospitalized in our hospital between September 2018 and October 2019. To observe different doses of glucocorticoid in the treatment of children with refractory mycoplasma pneumoniae pneumonia (RMPP), compare the differences of clinical manifestation, laboratory data, and radiological appearances between the three groups, provide predictive indicators for the severity of the disease and guide the dosage of glucocorticoids.

**Methods**

**Research Objects**

**Clinical information**

In this study we retrospectively collected the data of 279 patients with RMMP who hospitalized in our hospital between September 2018 and October 2019. A total of 23 children were excluded, of which 5 children were diagnosed with tuberculosis, 8 children were discharged shortly, and 10 children had used hormones before hospitalization, the remaining 256 children were divided into three subgroups: Group I was not given methylprednisolone, and 75 cases were included; group II was given methylprednisolone ≤125mg/d, and 115 cases were enrolled; group III was given methylprednisolone >125mg/d, and 66 cases were included.

**Diagnostic criteria**

MPP diagnostic criteria\[16\] Symptoms and signs of pneumonia on admission, including fever, cough, abnormal lung auscultation, etc; Chest radiograph reveals pneumonia; Positive results for serologic test (MP IgM positive and antibody titer≥ 1:160) and/or have the positive results for MP polymerase chain reaction (PCR) tests of nasopharyngeal secretions or blood. RMPP were defined as cases showing clinical and radiological deterioration despite appropriate antibiotic therapy for 7 days or more\[5,17,18\]

**Inclusion criteria**

(1) Meet the diagnostic criteria of MPP; (2) Meet the definition of RMPP; (3) Age <16 years old.

**Exclusion criteria**
1) Patients who had a history of tuberculosis, bronchiectasis, lung tumors; 2) Patients who had diseases such as severe malnutrition, unconsciousness, chronic cardiac and pulmonary disease, congenital disease, immunodeficiency; 3) Patients who received corticosteroids before admission; 4) Discharge within 8 hours after admission.

Glucocorticoid doses

According to study\[19-25\] the dosage of glucocorticoids is classified as follows: <7.5 mg prednisone equivalent a day is low dose; \(\geq 7.5\) mg, but <30 mg prednisone equivalent a day is medium dose; \(\geq 30\) mg, but <100 prednisone equivalent a day is high dose; \(\geq 100\) mg prednisone equivalent a day is very high dose; >250 mg prednisone equivalent a day for one or a few days is pulse therapy. Above 100 mg prednisone equivalent a day there is virtually a 100% receptor saturation with regard to cytosolic receptors.\[26\]. Therefore, a further increase in dose may exert rapid effects through non-genomic. In the genomic effect\[20, 22\] 100mg prednisolone is equivalent to 125mg methylprednisolone, so this article uses 125mg methylprednisolone as the grouping standard. Due to the combined effects of genomic and non-genomic effects\[22, 24\] methylprednisolone is a commonly used hormone in clinical practice.

Data collection

Demographic, clinical information, laboratory data and radiological findings were retrospectively collected from all children who were included in the study. Nasopharyngeal aspirate/swab specimens were routinely collected within 24 hours of admission. Respiratory specimens were tested for bacterial culture, virus using direct immunofluorescence assays and MP using PCR. Peripheral blood samples were collected upon admission for the determination of the complete blood count, C-reactive protein (CRP), lactate dehydrogenase (LDH), procalcitonin (PCT), interleukin (IL)-6, lactic acid (La), ferritin (Fer), d-dimer, fibrinogen (Fg), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and specific antibody to MP. Blood cultures was also performed on admission. All patients underwent imaging studies before admission or during hospitalization. During the hospitalization, we also evaluated the extra-pulmonary complications (liver function abnormalities, myocarditis, encephalitis, rash, proteinuria, hemolytic anemia and arthritis) of patients\[27\]. Hypoxia was defined as any recorded oxygen saturation of <92% by pulse oximetry, measured on room air\[28, 29\].

Observation indexes

Clinical features ((sex, age, duration of fever, duration of hospitalization, complications, hypoxia, etc.), radiological findings and laboratory data.

Ethics

The study was approved by the ethics committee of the Tianjin Children's Hospital. And the data from patients were analyzed anonymously.
Statistical Analysis

Statistical analyses were performed using SPSS software (version 22.0). Normal distribution data were expressed as mean ± SD (x ± s). Independent-Samples T-test or One-way ANOVA was used to compare these data. The skewed distribution data were expressed as the median values (P25, P75), which comparisons were made by the Mann-Whitney U-test. And Chi-squared tests were used to compare categorical data. Meanwhile, we use the laboratory indicators with significant differences as independent related risk factors to make the ROC curve, and use the area under the ROC curve (AUC) to reflect the accuracy of the diagnostic test. The value range of AUC is 0.5–1, AUC=0.5, which means a completely worthless diagnosis; 0.5<AUC≤0.7, which means low diagnostic accuracy; 0.7<AUC≤0.9, which indicates that the diagnostic accuracy is moderate; 0.9<AUC<1, which means higher diagnostic accuracy; AUC=1 indicates a perfectly ideal diagnosis. Take the point closest to the upper left corner of the ROC curve, which has the largest sum of sensitivity and specificity, as the optimal value of prediction. Multivariate logistic regression analysis was performed to select the variables associated with the severity of RMPP and glucocorticoid dosage. Statistical significance was defined as P<0.05.

Results

General information of patients

A total of 279 patients who were diagnosed with RMPP in our hospital from September 2018 to October 2019 were enrolled in the study. A total of 23 children were excluded, of which 5 children were diagnosed with tuberculosis, 8 children were discharged shortly, and 10 children had used hormones before hospitalization, and the remaining 256 patients were included in the study. All patients had positive results of PCR test and/or serological detection. All patients were treated with macrolide antibiotics. All patients were previously healthy, without underlying disease. According to the dose of glucocorticoid, the children were divided into three subgroups. 75 patients were in group I (36 females, 39 males), with the median age of 6 (4-8) years, with the median weight of 24.15 (18.20-28.18) kg; 115 patients were in group II (53 females, 62 males), with the median age of 6 (3-8) years, with the median weight of 18.55 (15.45-24.45) kg; 66 patients were in group III (30 females, 36 males), with the median age of 7 (5-8.25) years, with the median weight of 24.1 (19.88-30.55) kg, shown in Table 1. No difference in gender distribution was found between the three groups (P>0.05). The median age and weight of between the three groups were statistically significant (P<0.05).

Clinical information of patients

There were no statistically significant differences in fever and cough among the three groups (P>0.05). The incidence of hypoxemia was 59.1% in group III, 15.7% in group II and 0% in group I, with a significant difference (P<0.05) (Table 1). The incidence of respiratory failure was 6.1% in group III, 2.6% in group II and 0% in group I, with a statistically significant (P<0.05). Concerning the clinical course, we found that the median length of fever was 13 (10-15) days in group III, 11 (8-13) days in group II and 9 (6-11) in group I, with a statistically significant difference (P<0.05). The median length of stay days was 13 (10.75-
16) days in group III, 9 (8-11) days in group II and 6 (6-7) days in group I did reach statistical significance (P<0.05). The incidence of extrapulmonary complications was 87.9% in group III, 60.9% in group II and 16% in group I, with a significant difference (P<0.05). The incidence of Thromboembolism was 3% in group III, 1.7% in group II and 0% in group I did not reach statistical significance (P>0.05).

Management

All patients were treated with antibiotics. The median hormone dose was 200(163-380)mg/d in group III, 49(34-74)mg/d in group II and 0 in group I, with a significant difference (P<0.05). The use rate of gamma globulin was 40.9% in group III, 17.4% in group II and 0 in group I, the difference was statistically significant (P <0.05). The use rate of bronchoscopy was 93.9% in group III, 74.8% in group II and 52% in group I, with a statistically significant (P<0.05). The incidence of plastic bronchitis was 66.7% in group III, 21.7% in group II and 5.3% in group I, the difference was statistically significant (P <0.05).

Table 1. Clinical characteristic

| Clinical information | Group I (75) | Group II (115) | Group III (66) | P-value |
|----------------------|-------------|---------------|---------------|--------|
| General information  |             |               |               |        |
| Sex (female/male)    | 36/39       | 53/62         | 30/36         | 0.949  |
| Age, years           | 6;4-8;      | 6;3-8;        | 7;5-8.25;     | 0.014  |
| Weight, kg           | 24.15;18.2-28.18; | 18.55;15.45-24.45; | 24.1;19.88-30.55; | 0.001  |
| Clinical presentation, n (%) |           |               |               |        |
| Fever                | 70;93.3%    | 112;97.4%     | 66;100%       | 0.787  |
| Cough                | 69;92%      | 114;99.1%     | 65;98.5%      | 0.561  |
| Hypoxemia            | 0           | 18;15.7%      | 39;59.1%      | 0.001  |
| Respiratory failure  | 0           | 3;2.6%        | 4;6.1%        | 0.041  |
| Extra-pulmonary complications | 12;16%      | 70;60.9%      | 58;87.9%      | 0.000  |
| Thromboembolism      | 0           | 2;1.7%        | 2;3.0%        | 0.211  |
| Length of fever, days| 9;6-11;     | 11;8-13;      | 13;10-15;     | 0.000  |
| Length of stay, days | 6;6-7;      | 9;8-11;       | 13;10-15;     | 0.000  |
| Management           |             |               |               |        |
| Hormone dose, mg/d   | 0           | 49;34-74;     | 200;163-380;  | 0.000  |
| Gamma globulin, n (%)| 0           | 20;17.4%      | 27;40.9%      | 0.000  |
| Bronchoscopy, n (%)  | 39;52%      | 86;74.8%      | 62;93.9%      | 0.000  |
| Plastic bronchitis, n (%) | 4;5.3%      | 25;21.7%      | 44;66.7%      | 0.000  |

Data are presented as number (percentage), median (25th-75th percentile).

Laboratory data

Laboratory data in three groups of patients were summarized in Tables 2. The median values of Fg, PT, TT, La and AST did not differ significantly between the three groups. (P >0.05). The median levels of WBC, CRP, LDH, FER, D-dimer, APTT, PLT, PCT, IL-6, ALT, and the median percentage of peripheral neutrophils in children between groups I, II, and III showed a gradual upward trend, with a significant difference (P<0.05).

Table 2. Laboratory characteristic
Laboratory information

|                         | Group I (n=75) | Group II (n=115) | Group III (n=66) | P-value |
|-------------------------|---------------|------------------|------------------|---------|
| **White blood cell (×10⁹/L)** | 7.12(5.91-9.05) | 9.30(7.56-11.24) | 10.72(8.48-13.07) | 0.000   |
| Neutrophil, %           | 49.50(40.6-60.5) | 50.50(45.5-55.2) | 70.50(65.2-75.2) | 0.000   |
| Lymphocytes, %          | 38.20(30.6-46.5) | 21(16.5-34.2)    | 12.25(9.38-16.1)  | 0.000   |
| CRP, mg/L               | 16.70(5.3-24.1) | 45(14.5-68.5)    | 69.46-107.5       | 0.000   |
| LDH, IU/L               | 354(278-450)    | 467(354-626)     | 539(395-698)      | 0.000   |
| Fer, ng/L               | 119.7(90.98-174.8) | 266.4(143.9-373) | 467.3(298.6-742.25)| 0.000   |
| D-D, mg/L               | 0.3(0.2-0.4)    | 0.4(0.2-1.1)     | 0.8(0.2-2.2)      | 0.001   |
| PCT, g/l                | 3.697(3.22-4.40)| 3.674(2.94-4.38) | 3.855(3.20-4.70)  | 0.097   |
| PT                      | 11.17(11.3-12)  | 11.8(11.2-12.3)  | 11.65(10.98-12.03)| 0.621   |
| APTT                    | 31(29-34.9)     | 30.8(27.3-34)    | 27.40(24.08-31.48)| 0.005   |
| TT                      | 16.7(15.8-17.1) | 16.5(15.8-17.4)  | 17.20(16.30-17.83)| 0.089   |
| PLT                     | 378(329-467)    | 318(259-448)     | 299(235.7-398.75) | 0.002   |
| PCT, ng/ml              | 0.09(0.07-0.14) | 0.18(0.1-0.43)   | 0.34(0.17-0.77)   | 0.008   |
| IL-6, pg/ml             | 15.09(9.64-22.64)| 28.165(15.06-50.77)| 49.1(28.27-101.63)| 0.000   |
| La, mmol/L              | 2.61(2.25-3.38) | 2.76(2.163-3.39) | 2.87(2.27-3.37)   | 0.637   |
| AST, U/L                | 28(23-35)       | 36.5(29.5-51.25) | 37.5(28.7-52.25)  | 0.058   |
| ALT, U/L                | 14(12-17)       | 16(11.75-25)     | 20(12.75-46.75)   | 0.016   |

Data are presented as number (percentage).

Radiological findings

In addition to laboratory data, radiological manifestations were more severe in group III than that in group II and I (Table 3). Radiological findings in three groups of patients were summarized in Tables 3. The difference in the incidence of atelectasis (30.3% versus 23.5% versus 14.7%) and pleural thickening (62.1% versus 58.3% versus 61.3%) did not reach statistical significance (P>0.05). And there were statistically significant differences between the three groups in the incidence of pulmonary complications, including pulmonary consolidation (90.9% versus 60.9% versus 1.3%, P<0.05) and pleural effusion (43.9% versus 33.0% versus 13.3%, P<0.05).

Table 3. Radiological features

| Radiological features         | Group I (n=75) | Group II (n=115) | Group III (n=66) | P-value |
|-------------------------------|---------------|------------------|------------------|---------|
| Pulmonary consolidation, n (%)| 11(13.3%)     | 70(60.9%)        | 60(90.9%)        | 0.000   |
| Pleural effusion, n (%)       | 10(13.3%)     | 38(33.0%)        | 29(43.9%)        | 0.000   |
| Lobar atelectasis, n (%)      | 11(14.7%)     | 27(23.5%)        | 20(30.3%)        | 0.083   |
| Pleural thickening, n (%)     | 46(61.3%)     | 67(58.3%)        | 41(62.1%)        | 0.851   |

Data are presented as number (percentage).

Predictive values of the independent correlation factors in patients

WBC White blood cell, Neutrophil Peripheral neutrophils, Lymphocytes Peripheral Lymphocytes, CRP C-reactive protein, LDH Lactic dehydrogenase, Fer Ferritin, D-D D-dimer, Fg Fibrinogen, PCT Procalcitonin, IL-6 Interleukin (IL)-6, La Lactic acid, AST Aspartate aminotransferase, ALT Alanine aminotransferase.

Data are presented as median (25th-75th percentile).

WBC White blood cell, Neutrophil Peripheral neutrophils, Lymphocytes Peripheral Lymphocytes, CRP C-reactive protein, LDH Lactic dehydrogenase, Fer Ferritin, D-D D-dimer, Fg Fibrinogen, PCT Procalcitonin, IL-6 Interleukin (IL)-6, La Lactic acid, AST Aspartate aminotransferase, ALT Alanine aminotransferase.

Data are presented as median (25th-75th percentile).
To explore the optimal values of laboratory date for groups, receiver operator characteristic (ROC) curves were made and the cut-off values with maximum sensitivities and specificities were determined. Analysis of these ROC curves showed that WBC, neutrophil percentage, CRP, LDH, FER, PCT and IL-6 were significant predictors of the severity of RMPP and the dosage of glucocorticoids (Table 4). When the cut-off values for WBC, the percentage of neutrophil, CRP, LDH, FER, PCT and IL-6 were set at 7.7210^9/L, 63.3%, 32.05mg/L, 424.5IU/L, 231.15ug/ml, 0.145 ng/ml, 26.69pg/ml, respectively, Which were useful for differentiating the severity of RMPP and guiding glucocorticoid dosage. The sensitivity and specificity respectively were 61.3% and 77.7%, 82.7% and 78.3%, 89.3% and 69.7%, 72% And 62.9%, 89.3% and 69.7%, 81.3% and 67.4%, 84% and 62.9%.

Table 4. Predictive values of the independent correlation factors

| Independent factors | Cutoff value | Sensitivity | Specificity | AUC | P-value |
|---------------------|--------------|-------------|-------------|-----|---------|
| White blood cell (×10^9/L) | 7.720 | 0.613 | 0.777 | 0.735 | 0.000 |
| Neutrophil, % | 63.300 | 0.827 | 0.783 | 0.837 | 0.000 |
| CRP, mg/L | 32.050 | 0.893 | 0.697 | 0.831 | 0.000 |
| LDH, IU/L | 424.500 | 0.720 | 0.629 | 0.734 | 0.000 |
| FER, ng/L | 231.150 | 0.893 | 0.697 | 0.848 | 0.000 |
| PCT, ng/ml | 0.145 | 0.813 | 0.674 | 0.745 | 0.000 |
| IL-6, pg/ml | 26.690 | 0.840 | 0.629 | 0.770 | 0.000 |

AUC: area under the ROC curve; Cut-off value: the value on the ROC curve is closest to the upper right to take maximum sensitivity and specificity; P value: the AUC value of the independent factors compared to ROC curve reference value 0.5.

Multiple logistic regression analysis for the related factors

Multivariate logistic regression analysis was performed on the factors that predict the severity of RMPP and guide the dosage of glucocorticoids. The LDH 424.5IU/L, PCT 0.145 ng/ml, IL-6 26.69pg/ml and lung consolidation were significantly predictive regarding the differentiation between the three groups, with the odd ratio (OR) values of 2.301, 2.095, 1.009 and 1.053 in Table 5.

Table 5. Stepwise logistic regression analysis for the related factors

| Variable         | B   | S.E. | Wald  | P-value | OR    | 95%CI       | Lower | Upper |
|------------------|-----|------|-------|---------|-------|-------------|-------|-------|
|                  |     |      |       |         |       |             |       |       |
| LDH, IU/L        | 2.116 | 0.617 | 11.775 | 0.001 | 2.301 | 1.478 | 2.803 |
| IL-6, pg/ml      | 1.628 | 0.617 | 6.996  | 0.008 | 2.095 | 1.521 | 2.069 |
| PCT, ng/ml       | 1.102 | 0.558 | 3.894  | 0.048 | 1.009 | 1.007 | 2.987 |
| Pulmonary         | 4.771 | 1.176 | 16.469 | 0.000 | 1.053 | 1.785 | 2.540 |

Discussion
Mycoplasma pneumoniae infection was the leading pathogen of CAP in children. It is traditionally assumed that MP infection was a benign process, but more and more severe cases have been reported in recent years\cite{30-34}. Cases of RMPP were reported increasingly, which displayed clinical and radiological progression after macrolide therapy for 7 days or longer\cite{5,15,35}. Thus, it is essential for pediatricians to identify RMPP as early as possible, prompt therapy and prevent disease progression. Because the influence of pathogenesis, the majority of RMPP will produce both intrapulmonary symptoms and extrapulmonary complications, which may involve the heart system, liver system, central nervous system, hematopoietic system and skin.

A number of studies have suggested that humoral and cellular immune responses\cite{36,37} contribute to the pathogenesis of MP infection, providing a theoretical basis for the application of immunosuppressive agents such as glucocorticoids in mycoplasma pneumonia. Studies have shown that the addition of GCs on the basis of the conventional treatment has a definite effect on RMPP, which is conducive to control the disease progression, improve the condition and reduce sequelae\cite{6,38}. So, it is a very important for pediatricians to study the application of glucocorticoids in the treatment of RMPP\cite{5,6,39}. At the moment, retrospective, observational study, 279 patients with RMPP were enrolled. According to the inclusion criteria and the exclusion criteria of RMPP\cite{2,6,17-19}, 23 cases were excluded, 256 cases were included. Among them, there are 75 cases in group I, 115 cases in group II, and 66 cases in group III. The different clinical characteristics between the RMPP patients were compared. First of all, this study found that the median age of children in group III was older than that in group II and I (7 (5-8.25), 6 (3-8), 6 (4-8), P<0.05), which was consistent with the previous reports\cite{3,15}. Children's immune system develops more mature with age, and is prone to excessive inflammatory reaction to MP, which may lead to the progression of RMPP. We also found that the median weight of children in group III was larger than that in groups II and I (24.1 (19.88-30.55), 18.55 (15.45-24.45), 24.15 (18.2-28.18), P<0.05), which may be because the greater the body weight, the greater the hormone dose. Secondly, higher incidence of hypoxemia, extra-pulmonary complications and plastic bronchitis were found in the group III than those in the groups II and I (P<0.05). Higher incidence of hypoxemia, extra-pulmonary complications and plastic bronchitis were found in the group II than those in the groups I (P<0.05). Moreover, the proportion of patients required oxygen therapy, gamma globulin and bronchoscopy in the group III was higher than that in the group II and I (P<0.05). The proportion of patients required oxygen therapy, gamma globulin and bronchoscopy in the group II was higher than that in the group I (P<0.05). We found that patients with group III showed longer median length of fever days, longer median length of stay days and higher median hormone dose than those of patients with group II and I (P<0.05). We also found that patients with group II showed longer median length of fever days, longer median length of stay days and higher median hormone dose than those of patients with group I (P<0.05). All these results indicated that if not promptly treated, this can lead to severe deterioration. It also suggested that more severe of symptoms, the longer of clinical course, the greater of hormone use, and higher incidence of extrapulmonary complications, higher incidence of plastic bronchitis. Additionally, our research also showed that higher incidence of pulmonary consolidation and pleural effusion were found in group III than in groups II and I (P<0.05), higher incidence of pulmonary consolidation and pleural effusion were found in group II than in groups I.
(P<0.05). The diversification of imaging findings may be due to direct microbial effects and strong immune inflammatory response. Finally, the study implied that the median levels of WBC, CRP, LDH, FER, D-dimer, APTT, PLT, PCT, IL-6, ALT, and the median percentage of peripheral neutrophils in children between groups I, II, and III showed a gradual upward trend (P<0.05). Appropriate treatment measures should be taken when laboratory markers were higher.

To investigate potential factors that may predict the severity of RMPP and guide the dosage of glucocorticoids, we analyzed some laboratory indexes, which were significantly different between the three groups using ROC curve. This study found that the area under the curve of seven independent factors, including WBC, the percentage of neutrophil, CRP, LDH, FER, PCT, and IL-6 were above 0.7 in ROC curve analysis, indicating fair discriminative power for predicting RMPP. The optimal cutoff value for these seven factors was 7.72109/L, 63.3%, 32.05mg/L, 424.5IU/L, 231.15ug/L, 0.145ng/ml, 26.69pg/ml, respectively. In addition, basing on the cutoff values of these seven factors and significant radiological findings, multiple logistic regression analysis was performed to improve the accuracy of prediction. We found that LDH 424.5IU/L, PCT 0.145ng/ml, IL-6 26.69pg/ml and lung consolidation may be significant predictors for severity of disease and dose of glucocorticoids in RMPP. LDH was associated with many lung diseases, such as obstructive and interstitial lung diseases \[40, 41\]. Several studies \[1, 3, 42, 43\] also found that serum LDH was elevated in RMPP. In our study, we found that the area under the curve of LDH was 0.734 in ROC curve analysis, showing fair discriminative power for predicting RMPP. The optimal cutoff for LDH was 424.5IU/L, the sensitivity was 72\% and the specificity was 62.9\%, which was similar with the previous reports \[42, 43\]. And stepwise logistic regression analyzed that the serum LDH (odds ratio of 2.301, 95% CI 1.478-2.803, P = 0.001) was significant meaningful. The reason for the difference in studies may be the occurrence of mixed infection events that we could not identify. PCT plays an important role in immune response. In our study, we found that the area under the curve of PCT was 0.745 in ROC curve analysis, showing fair discriminative power for predicting RMPP. The optimal cutoff for PCT was 0.145ng/ml, the sensitivity was 81.3% and the specificity was 67.4%. And stepwise logistic regression analyzed that the serum PCT (odds ratio of 1.009, 95% CI 1.007-2.987, P = 0.048) was significant meaningful. At present, it is believed that the increase in PCT is related to the severity of disease, further suggesting that there may be an overactive immune response in RMPP. IL-6 is essential for the early phase of the immune response. In our research, we found that the area under the curve of IL-6 was 0.770 in ROC curve analysis, showing fair discriminative power for predicting RMPP. The optimal cutoff for IL-6 was 26.69pg/ml, the sensitivity was 84% and the specificity was 62.9%. And stepwise logistic regression analyzed that the serum IL-6 (odds ratio of 2.095, 95% CI 1.521-2.069, P = 0.008) was significant meaningful. Chen et al showed that the optimal value of IL-6 was 14.75pg/ml in RMPP \[43\]. Furthermore, it has been suggested that the increase in IL-6 is associated with the severity of the disease and the duration of disease period \[44\], which further indicates that there may be an excessive immune response in RMPP. Glucocorticoids combined with antibiotics can reduce inflammation and have a significant effect, which has been demonstrated in several studies \[3-7\].
However, there are different opinions on the dosage of hormones in the existing domestic and foreign literatures\cite{3,4,6,45}. You and Lee et al.\cite{46} used methylprednisolone 10mg/kg/d(intravenous infusion 2 ~ 3d and suspension reduction within 1 week) or gamma globulin 1g/kg/d (1 ~ 2 times) for a small percentage of patients who failed oral treatment. All patients showed significant improvement in clinical and radiological manifestations within a few days without associated side effects. Lee\cite{3} et al. gave prednisolone 1mg/kg/d orally to 15 children with RMPP, and reduced the dosage for 7d after continuous administration for 3-7d, which showed obvious effect in the treatment of children with RMPP. Luo et al. prospectively demonstrated that oral prednisone (2mg/kg/d) was more effective than azithromycin alone in children with RMPP. And Tamura\cite{5} on 6 patients with RMPP applications such as methylprednisolone 30 mg/kg/d continuous intravenous drip in 3 d, all of the patients in body temperature returned to normal within 14 h, clinical symptoms improved significantly, they think that compared with conventional therapy, combined use of hormone treatment, can reduce the length of stay, reduce the happening of the RMPP, and no hormone adverse reactions. The study implied that older children are more prone to leading to more severe presentations, higher incidence of extra-pulmonary complications and more serious radiological findings. LDH 424.5IU/L, PCT 0.145ng/ml, IL-6 26.69pg/ml and pulmonary consolidation can predict the severity of RMPP and guide hormone dose, which can aid in early recognition of children with severe illness, use appropriate dosage of hormone, and reduce sequelae.

This study indicated that in the treatment of refractory mycoplasma pneumonia, timely use of appropriate doses of glucocorticoids can reduce the intensity of local inflammation, alleviates the immune reaction, and promote disease recovery. Therefore, under effective anti-infective therapy, glucocorticoid is effective, safe and convenient, and significantly speeds up the healing process, which is a vital means for the treatment of refractory mycoplasma pneumonia. During the treatment of RMPP with glucocorticoid, blood pressure, blood glucose, blood potassium and liver function should be monitored, and observation for adverse reactions such as circulatory system and gastrointestinal bleeding, pay attention to ECG monitoring during shock dose treatment. And pay attention to: suitable time; exclude whether there are other infections or lesions; prevent the occurrence of double infection. The study has some limitations. Firstly, it was a retrospective study, therefore there may have been some selection bias, so a large number of children with RMPP are need to be recruited and further prospective studies are needed to be carried out. Secondly, the distribution of patients between the three groups is not matching, which may affect the statistical results. Thirdly, the patients come from the same region, the risk factors associated with glucocorticoid resistance may not be applicable to patients in other regions, requiring multicenter studies in the future. Fourth, our hospital is a tertiary hospital with many critically ill patients. The uneven distribution of critically ill patients in this study has a certain impact on the experimental results. Fifth, there may be some cases in which the patients had a combined MP and other pathogens infection which cannot be detected, which might result in RMPP. Finally, the optimal value of risk factor obtained by ROC curve may have some limitations and only guide judgment to a certain extent. More clinical data should be accumulated and further verified in clinical work for a more accurate reference standard.
Conclusion

Our study suggests that excessive immune inflammatory response may play an important role in RMPP. LDH 424.5IU/L, PCT 0.145ng/ml and IL-6 26.69pg/ml and lung consolidation are predictors for predicting the severity of RMPP and guiding the dose of glucocorticoids.

Abbreviation

MP: Mycoplasma Pneumoniae; CAP: Community Acquired Pneumonia; RMPP: Refractory mycoplasma pneumoniae pneumonia; PCR: Polymerase chain reaction; CRP: C-reactive protein; LDH: Lactate dehydrogenase; PCT: Procalcitonin; IL: Interleukin; La: lactic acid; Fer: Ferritin; D-D: D-dimer; Fg: Fibrinogen; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ROC: Receiver operating characteristic

Declarations

Acknowledgments

We owe our thanks to Wei Guo, Yaoyao Ling, and Jiao Tian for their work on revising and data extracting in this manuscript.

Authors’ contributions

Conception and Design: ZLZ, TQZ, and YSX; Extraction of Data: ZLZ, WG, YYL, and JT; Drafting the Article: ZLZ; Revising It for Intellectual Content: ZLZ, and YSX; Final Approval of the Completed Article: ZLZ, TQZ, and YSX. All authors read and approved the final manuscript.

Funding

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were following the Ethics Committee of Tianjin Children's Hospital. This article does not contain any studies with animals performed by any of the authors. The data used in this study were anonymized before its use.

Consent for publication

Not applicable.
Competing interests

The authors declare no conflict of interest.

Author details

1Tianjin Medical University, No.22, Qixiangtai Road, Heping District, Tianjin 300070, China. 2Department of Respiratory, The Children's Hospital of Tianjin (Children's Hospital of Tianjin University), Tianjin, China.

References

[1] Liu JR, Peng Y, Yang HM, Li HM, Zhao SY, Jiang ZF. [Clinical characteristics and predictive factors of refractory Mycoplasma pneumoniae pneumonia]. Zhonghua Er Ke Za Zhi. 2012. 50(12): 915-8.

[2] Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. Clin Microbiol Rev. 2004. 17(4): 697-728, table of contents.

[3] Lee KY, Lee HS, Hong JH, et al. Role of prednisolone treatment in severe Mycoplasma pneumoniae pneumonia in children. Pediatr Pulmonol. 2006. 41(3): 263-8.

[4] Miyashita N, Obase Y, Ouchi K, et al. Clinical features of severe Mycoplasma pneumoniae pneumonia in adults admitted to an intensive care unit. J Med Microbiol. 2007. 56(Pt 12): 1625-9.

[5] Tamura A, Matsubara K, Tanaka T, Nigami H, Yura K, Fukaya T. Methylprednisolone pulse therapy for refractory Mycoplasma pneumoniae pneumonia in children. J Infect. 2008. 57(3): 223-8.

[6] Lu A, Wang L, Zhang X, Zhang M. Combined treatment for child refractory Mycoplasma pneumoniae pneumonia with ciprofloxacin and glucocorticoid. Pediatr Pulmonol. 2011. 46(11): 1093-7.

[7] Radisic M, Torn A, Gutierrez P, Defranchi HA, Pardo P. Severe acute lung injury caused by Mycoplasma pneumoniae: potential role for steroid pulses in treatment. Clin Infect Dis. 2000. 31(6): 1507-11.

[8] Wang J, Yang Y, Zhao SY. [Bronchitis obliterans in children: report of two cases and literature review]. Zhonghua Er Ke Za Zhi. 2010. 48(10): 764-6.

[9] Sun LL, Ye C, Zhou YL, Zuo SR, Deng ZZ, Wang CJ. Meta-analysis of the Clinical Efficacy and Safety of High- and Low-dose Methylprednisolone in the Treatment of Children With Severe Mycoplasma Pneumoniae Pneumonia. Pediatr Infect Dis J. 2020. 39(3): 177-183.

[10] Yan C, Xue G, Zhao H, et al. Molecular and clinical characteristics of severe Mycoplasma pneumoniae pneumonia in children. Pediatr Pulmonol. 2019. 54(7): 1012-1021.

[11] Garcia AV, Fingeret AL, Thirumoorthi AS, Kadenhe-Chiweshe A, Kandel JJ. Severe Mycoplasma pneumoniae infection requiring extracorporeal membrane oxygenation with concomitant ischemic stroke in a child. Pediatr Pulmonol. 2013. 48(1): 98-101.
[12] Park IH, Choi dY, Oh YK, Kim JD, Yu ST. A case of acute myopericarditis associated with Mycoplasma pneumoniae infection in a child. Korean Circ J. 2012. 42(10): 709-13.

[13] Xin D, Mi Z, Han X, et al. Molecular mechanisms of macrolide resistance in clinical isolates of Mycoplasma pneumoniae from China. Antimicrob Agents Chemother. 2009. 53(5): 2158-9.

[14] Okada T, Morozumi M, Tajima T, et al. Rapid effectiveness of minocycline or doxycycline against macrolide-resistant Mycoplasma pneumoniae infection in a 2011 outbreak among Japanese children. Clin Infect Dis. 2012. 55(12): 1642-9.

[15] Wang M, Wang Y, Yan Y, et al. Clinical and laboratory profiles of refractory Mycoplasma pneumoniae pneumonia in children. Int J Infect Dis. 2014. 29: 18-23.

[16] Lee SC, Youn YS, Rhim JW, Kang JH, Lee KY. Early Serologic Diagnosis of Mycoplasma pneumoniae Pneumonia: An Observational Study on Changes in Titers of Specific-IgM Antibodies and Cold Agglutinins. Medicine (Baltimore). 2016. 95(19): e3605.

[17] Subspecialty Group of Respiratory Diseases, The Society of Pediatrics, Chinese Medical Association The Editorial Board, Chinese Journal of Pediatrics. [Guidelines for management of community acquired pneumonia in children(the revised edition of 2013) (II)]. Zhonghua Er Ke Za Zhi. 2013. 51(11): 856-62.

[18] Ding Y, Chu C, Li Y, et al. High expression of HMGB1 in children with refractory Mycoplasma pneumoniae pneumonia. BMC Infect Dis. 2018. 18(1): 439.

[19] Huscher D, Thiele K, Gromnica-Ihle E, et al. Dose-related patterns of glucocorticoid-induced side effects. Ann Rheum Dis. 2009. 68(7): 1119-24.

[20] Buttgereit F, da Silva JA, Boers M, et al. Standardised nomenclature for glucocorticoid dosages and glucocorticoid treatment regimens: current questions and tentative answers in rheumatology. Ann Rheum Dis. 2002. 61(8): 718-22.

[21] Buttgereit F, Wehling M, Burmester GR. A new hypothesis of modular glucocorticoid actions: steroid treatment of rheumatic diseases revisited. Arthritis Rheum. 1998. 41(5): 761-7.

[22] Piper BJ, Alinea AA, Wroblewski JR, et al. A Quantitative and Narrative Evaluation of Goodman and Gilman's Pharmacological Basis of Therapeutics. Pharmacy (Basel). 2019. 8(1).

[23] Buttgereit F, Scheffold A. Rapid glucocorticoid effects on immune cells. Steroids. 2002. 67(6): 529-34.

[24] Schmid D, Burmester GR, Tripmacher R, Kuhnke A, Buttgereit F. Bioenergetics of human peripheral blood mononuclear cell metabolism in quiescent, activated, and glucocorticoid-treated states. Biosci Rep. 2000. 20(4): 289-302.
[25] Lipworth BJ. Therapeutic implications of non-genomic glucocorticoid activity. Lancet. 2000. 356(9224): 87-9.

[26] Wu WC, Su DH, Chiu WY, et al. Status of endocrinology and metabolism specialists in Taiwan. J Formos Med Assoc. 2020.

[27] Zhou Y, Zhang Y, Sheng Y, Zhang L, Shen Z, Chen Z. More complications occur in macrolide-resistant than in macrolide-sensitive Mycoplasma pneumoniae pneumonia. Antimicrob Agents Chemother. 2014. 58(2): 1034-8.

[28] Tan TQ, Mason EO Jr, Wald ER, et al. Clinical characteristics of children with complicated pneumonia caused by Streptococcus pneumoniae. Pediatrics. 2002. 110(1 Pt 1): 1-6.

[29] Izumikawa K, Izumikawa K, Takazono T, et al. Clinical features, risk factors and treatment of fulminant Mycoplasma pneumoniae pneumonia: a review of the Japanese literature. J Infect Chemother. 2014. 20(3): 181-5.

[30] Kim CK, Kim SW, Kim JS, et al. Bronchiolitis obliterans in the 1990s in Korea and the United States. Chest. 2001. 120(4): 1101-6.

[31] Wang RS, Wang SY, Hsieh KS, et al. Necrotizing pneumonitis caused by Mycoplasma pneumoniae in pediatric patients: report of five cases and review of literature. Pediatr Infect Dis J. 2004. 23(6): 564-7.

[32] Azumagawa K, Kambara Y, Murata T, Tamai H. Four cases of arthritis associated with Mycoplasma pneumoniae infection. Pediatr Int. 2008. 50(4): 511-3.

[33] Hawkins S, Rausch CM, McCanta AC. Constrictive pericarditis secondary to infection with Mycoplasma pneumoniae. Curr Opin Pediatr. 2011. 23(1): 126-9.

[34] Khan FY, A yM. Mycoplasma pneumoniae associated with severe autoimmune hemolytic anemia: case report and literature review. Braz J Infect Dis. 2009. 13(1): 77-9.

[35] Luo Z, Luo J, Liu E, et al. Effects of prednisolone on refractory mycoplasma pneumoniae pneumonia in children. Pediatric PulmonologyPediatric PulmonologyPediatric PulmonologyPediatric Pulmonology. 2014. 49(4): 377-380.

[36] Kim NH, Lee JA, Eun BW, et al. Comparison of polymerase chain reaction and the indirect particle agglutination antibody test for the diagnosis of Mycoplasma pneumoniae pneumonia in children during two outbreaks. Pediatr Infect Dis J. 2007. 26(10): 897-903.

[37] Attilakos A, Palaiologou P, Lagona E, Voutsioti A, Dinopoulos A. Mycoplasma pneumoniae encephalopathy: recovery after intravenous immunoglobulin. Pediatr Neurol. 2008. 38(5): 357-9.

[38] Luo Z, Luo J, Liu E, et al. Effects of prednisolone on refractory mycoplasma pneumoniae pneumonia in children. Pediatr Pulmonol. 2014. 49(4): 377-80.
[39] Chen L, Liu J, Zhao S, Yang Y, Wu J. [Clinical features and treatment of refractory Mycoplasma pneumoniae pneumonia unresponded to conventional dose methylprednisolone in children]. Zhonghua Er Ke Za Zhi. 2014. 52(3): 172-176.

[40] Drent M, Cobben NA, Henderson RF, Wouters EF, van Dieijen-Visser M. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. Eur Respir J. 1996. 9(8): 1736-42.

[41] Nakajima M, Kawahara Y, Yoshida K, Miyashita N, Niki Y, Matsushima T. Serum KL-6 as a possible marker for amiodarone-induced pulmonary toxicity. Intern Med. 2000. 39(12): 1097-100.

[42] Inamura N, Miyashita N, Hasegawa S, et al. Management of refractory Mycoplasma pneumoniae pneumonia: utility of measuring serum lactate dehydrogenase level. J Infect Chemother. 2014. 20(4): 270-3.

[43] Zhang Y, Zhou Y, Li S, Yang D, Wu X, Chen Z. The Clinical Characteristics and Predictors of Refractory Mycoplasma pneumoniae Pneumonia in Children. PLoS One. 2016. 11(5): e0156465.

[44] Tian F, Han B, Duan M. [Serum tumor necrosis factor-α, interleukin-6 and galctin-3 concentrations in children with Mycoplasma pneumoniae pneumonia]. Zhongguo Dang Dai Er Ke Za Zhi. 2014. 16(10): 1001-4.

[45] Cimolai N. Corticosteroids and complicated Mycoplasma pneumoniae infection. Pediatr Pulmonol. 2006. 41(10): 1008-9; author reply 1010.

[46] Youn Y, Lee K. Mycoplasma pneumoniae pneumonia in children. Korean J Pediatr. 2012. 55(2): 42-47.