Evaluation on blood coagulation and C-reactive protein level among children with mycoplasma pneumoniae pneumonia by different chest imaging findings

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Abstract
Mycoplasma pneumoniae infection may induce a systemic hypercoagulable abnormality, like organ embolism and infarction. Indexes of blood coagulation and C-reactive protein (CRP) have been reported different between healthy people and mycoplasma pneumoniae pneumonia (MPP) patients, but this difference in MPP patients with different chest imaging findings has rarely been reported.

We performed a retrospective study of 101 children with MPP and 119 controls, combined with radiological examination and blood tests, to compare the blood coagulation and CRP level among MPP children with different chest imaging findings.

For the MPP children with different chest imaging findings, there were significant differences in CRP, fibrinogen (FIB) and D-dimer (D-D) levels among subgroups ($P = .004$, $P = .008$ and $P < .001$ respectively). The CRP level in group of interstitial pneumonia was significantly higher than that in groups of bronchopneumonia and hilar shadow thickening ($P = .003$ and $P = .001$ respectively). And the FIB and D-D values in group of lung consolidation were significantly higher than that in the other 3 groups (all $P < .05$). When compared with controls, the white blood cell, CRP, FIB, and D-D levels in MPP children were significantly higher, and the activated partial thromboplastin time and thrombin time levels were significantly lower (all $P < .05$).

Our results showed that CRP level changed most significantly in group of interstitial pneumonia, whereas FIB, D-D levels changed most significantly in the lung consolidation group.

Abbreviations: ALT $=$ alanine transaminase, APTT $=$ activated partial thromboplastin time, CAP $=$ community-acquired pneumonia, CRP $=$ C-reactive protein, D-D $=$ D-dimer, FIB $=$ fibrinogen, MP $=$ Mycoplasma pneumoniae, MPP $=$ mycoplasma pneumoniae pneumonia, PT $=$ prothrombin time, TT $=$ thrombin time.

Keywords: blood coagulation, chest imaging findings, C-reactive protein, mycoplasma pneumoniae pneumonia

1. Introduction
Community-acquired pneumonia (CAP) is a common and sometimes lethal infection. Although its therapeutic options are multiplying, CAP remains a significant cause of morbidity and mortality among children worldwide. Mycoplasma pneumoniae (MP) is 1 of the most prevalent pathogens causing CAP in children.[1-3] Infection by MP accounts for 15% to 20% of all CAP cases worldwide.[4] Epidemiological studies in Japan have demonstrated that MP is the second powerful cause of CAP, just next to streptococcus pneumoniae.[5]
MP binds to the cilia with the help of P1 protein, multiplies in the respiratory epithelial layer, and stimulates the respiratory tract to produce proinflammatory cytokines such as IL-6, C-reactive protein (CRP), a process leading to acute cellular inflammatory reaction and airway damage. Hence, the main affected organ is lung. MP pneumonia (MPP) is clinically characterized by dry cough, fever and general fatigue. The chest imaging of MPP children include consolidation, atelectasis, ground glass opacity and pleural effusion.

Nowadays, concerns have been raised about the effect of MP infection on extrapulmonary systems in children, including cardiovascular, neurologic, hematologic, dermatologic and hepatobiliary systems. In some cases, these effects can bring about severe, even life-threatening pneumonia. Koletsky and Weinstein reviewed eleven cases of fatal MP infection, only to find 1 with pneumonia. Surprisingly, abnormal blood coagulation and vascular thrombosis developed in 5 patients. Thus, we speculated that MP infection may induce a systemic hypercoagulable abnormality, like organ embolism and infarction. These cases have been reported recently, such as cerebral infarction, pulmonary embolism and splenic infarct. Indexes of blood coagulation and CRP have been reported different between healthy people and MPP patients, but this difference in MPP coagulation and CRP have been reported different between children with MPP and controls (Table 2). For the MPP children with different chest imaging findings showed that 17 cases were lung consolidation, 17 cases were interstitial pneumonia, 46 cases were bronchopneumonia, and 21 cases were hilar shadow thickening.

We excluded children if they fit the following criteria:

1. Children with haematological diseases or coagulant functional abnormality;
2. Children who took medicine that could affect the coagulation before admission
3. Children with a history of trauma or surgery
4. Children with lung malformation and diseases such as asthma;
5. Children with serious cardiac, hepatic and renal insufficiency;
6. Children with incomplete clinical data;
7. Children with other infections;
8. Children whose chest images were complex and varied as the disease continued.

At 7–10 days after pneumonia symptoms appeared, 2mL of venous blood was collected. With this blood, 0.1mL of serum was extracted, centrifuged, and transferred into reagent strips. Quantitative enzyme-linked immunosorbent assays were established to measure the levels of anti-MP IgM antibodies using IgM enzyme-linked immunosorbent assays kits (YHLO BIOTECH CO, LTD, Shenzhen China). The assay was considered positive if the level of IgM ≥ 1.1 mg.

For each child with MPP, 2 mL of venous blood was collected and sampled using BD tube (America) before treatment. After an interval of 60 minute, the blood sample was centrifuged at 3000r/min for 5 minutes in a Xiang Yi L355-1 centrifuge (Xiang Yi laboratory instrument development co, LTD, Hunan, China) and the plasma was immediately separated. During the following 2h, the coagulation status was evaluated with several indexes detected by the ACLTOP700 automatic blood coagulation analyser (Beckman Coulter), including prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), plasma fibrinogen (FIB) and D-dimer (D-D) levels. Other clinical indexes, such as white blood cell, CRP, platelet count, alanine transaminase (ALT) were analyzed with routine methods.

Meanwhile, 119 non-infectious inguinal hernia patients in pediatric surgery department of our hospital during the corresponding period were enrolled as the control group. The exclusion criteria were the same as that of the MPP group. Measurement of blood coagulation and anti-MP IgM antibody were conducted before surgery. Anti-MP IgM antibody in the control group was assured negative.

SPSS 20.0 (IBM Corp, Armonk, NY) was used for statistical analysis. Continuous data were shown as mean ± standard deviation (x ± s). All continuous data were tested for normality and homogeneity of variance. Analysis of variance or the Student t-test was used to determine differences of continuous variables between the groups. Least Significance Difference - t test method was used to identify significant differences between group means. Categorical data were shown as frequencies. Pearson chi-square test was used to analyze differences between categorical variables. Correlation analysis was used to detect the relationship between indexes. P < .05 was considered statistically significant.

3. Results

In total, 39 children were excluded due to ineligibility. Finally, 101 children with MPP (aged from 9 months to 12 years) and 119 controls (aged from 3 years to 14 years) were included in this study. Chest imaging findings showed that 17 cases were lung consolidation, 17 cases were interstitial pneumonia, 46 cases were bronchopneumonia, and 21 cases were hilar shadow thickening. The general characteristics and laboratory tests of children with MPP and controls are shown in Table 1. For the MPP children with different chest imaging findings, there were significant differences in age, height, weight and CRP levels among subgroups (P=0.001, P=0.007, P < .001 and P = .004 respectively). When compared with controls, the white blood cell and CRP levels in MPP children were significantly higher (P=0.007 and P < .001 respectively). However, no significant differences were found in platelet and ALT between groups.

Then we compared the blood coagulation indexes among MPP children and controls (Table 2). For the MPP children with different chest imaging findings, there were significant differences in FIB and D-D levels among subgroups (P=.008 and P < .001.
Comparison of blood coagulation among children with mycoplasma pneumoniae pneumonia and controls.

The general characteristics and laboratory tests of children with mycoplasma pneumoniae pneumonia and controls.

Indexes

| Variables | Lung consolidation (n=17) | Interstitial pneumonia (n=17) | Bronchopneumonia (n=46) | Hilar shadow thickening (n=21) | All cases (n=101) | Controls (n=119) |
|-----------|--------------------------|-----------------------------|------------------------|-----------------------------|-----------------|-----------------|
| Gender (boy) | 9 (52.9%) | 9 (52.9%) | 21 (45.7%) | 15 (71.4%) | 54 (53.5%) | 77 (64.2%) |
| Age (yr) | 6.59±3.46 | 3.99±1.81 | 4.58±2.14 | 3.55±1.50 | 4.60±2.43 | 5.77±2.50 |
| Height (cm) | 119.59±23.94 | 105.53±13.65 | 108.57±17.65 | 99.57±12.92 | 108.04±18.28 | 117.29±17.65 |
| Weight (kg) | 26.18±11.80 | 16.47±4.11 | 19.14±7.19 | 16.71±4.85 | 19.37±7.99 | 22.27±6.70 |
| B10 (10^9/L) | 9.86±3.93 | 10.36±5.34 | 8.96±3.45 | 8.61±4.42 | 9.27±4.09 | 8.02±2.29 |
| PT (PT) | 277.94±76.24 | 232.94±76.14 | 257.57±85.45 | 236.05±74.96 | 252.38±80.69 | 268.39±65.74 |
| CRP (mg/L) | 21.90±18.02 | 34.62±54.82 | 12.31±13.84 | 6.16±6.87 | 16.40±26.83 | 5.42±8.77 |
| ALT (U/L) | 22.82±20.33 | 14.12±4.69 | 16.82±14.43 | 15.38±5.00 | 17.08±13.26 | 18.40±9.96 |

Table 1: The general characteristics and laboratory tests of children with mycoplasma pneumoniae pneumonia and controls.

Cases

N| Lungs| Interstitial Pneumonia| Bronchopneumonia| Hilar Shadow Thickening| All Cases| Controls| p<0.05| p<0.01|
---|---|---|---|---|---|---|---|---|
Gender (boy)| 9 (52.9%)| 9 (52.9%)| 21 (45.7%)| 15 (71.4%)| 54 (53.5%)| 77 (64.2%)| .000| .001|
Age (yr)| 6.59±3.46| 3.99±1.81| 4.58±2.14| 3.55±1.50| 4.60±2.43| 5.77±2.50| .001| .001|
Height (cm)| 119.59±23.94| 105.53±13.65| 108.57±17.65| 99.57±12.92| 108.04±18.28| 117.29±17.65| <.001| .007|
Weight (kg)| 26.18±11.80| 16.47±4.11| 19.14±7.19| 16.71±4.85| 19.37±7.99| 22.27±6.70| .011| <.001|
B10 (10^9/L)| 9.86±3.93| 10.36±5.34| 8.96±3.45| 8.61±4.42| 9.27±4.09| 8.02±2.29| .007| .501|
PT (s)| 277.94±76.24| 232.94±76.14| 257.57±85.45| 236.05±74.96| 252.38±80.69| 268.39±65.74| .112| .293|
CRP (mg/L)| 21.90±18.02| 34.62±54.82| 12.31±13.84| 6.16±6.87| 16.40±26.83| 5.42±8.77| <.001| .004|
ALT (U/L)| 22.82±20.33| 14.12±4.69| 16.82±14.43| 15.38±5.00| 17.08±13.26| 18.40±9.96| .112| .222|

p<0.01 for the comparison of all cases with controls; p<0.05 for the comparison among children with mycoplasma pneumoniae pneumonia by different chest imaging findings; ALT+ = alanine transaminase, CRP = C-reactive protein, PT = prothrombin time, TT = thrombin time.

4. Discussion

Unlike S pneumoniae that may directly infect the alveolar lumen, MP can infect the entire airway, even the interstitial lung and alveoli. MP infection may cause acute bronchiolitis with edema, bronchial wall ulcer, and perivascular or peribronchial interstitial opacities, including lymphocytes, plasma cells and macrophages.[14,15] Given these differences in MP infection at different regions, the imaging findings of MP may vary.[16] MP has various extrapulmonary manifestations. Among them, abnormal blood coagulation is drawing increasing attention in recent years. Also, the relationship between blood coagulation indexes and pro-inflammatory factors such as IL-6 and CRP, has also become a research focus.[17,18] In this study, we found significant differences in CRP and blood coagulation indexes among groups with different chest imaging findings. These results suggested that pulmonary and extrapulmonary manifestations might be closely related and could be shown by some indexes. Our aim was to find the relationship between chest imaging results and blood coagulation. Once this relationship is clarified, clinicians can simply know whether the patients have the risk of hemagglutination or even embolism according to the manifestation of chest X-ray, and take relevant examination and intervention treatment as early as possible to reduce the occurrence of serious complications and improve the long-term prognosis. However, as none of the patients have thrombosis or embolism in the study, our results do not show that CRP and blood coagulation indexes are related with thrombosis and embolism. Thus, further studies on these correlations are needed.

In this study, the age, height and weight of children with lung consolidation were significantly higher than those of other subgroups. This phenomenon can be often encountered in clinical practice. MP is a culprit of respiratory infection in school-age children.
Table 3

Multiple comparisons of CRP, FIB, and D-dimer in case groups.

| Comparison                          | CRP (mg/L) | FIB (g/L) | D-D (mg/L) | P    |
|-------------------------------------|------------|-----------|------------|------|
| Lung consolidation vs Intersitial pneumonia | 0.149      | 0.031     | 0.001      |      |
| Lung consolidation vs Bronchopneumonia | 0.187      | 0.002     | < 0.001    |      |
| Lung consolidation vs Hilar shadow thickening | 0.061      | 0.002     | < 0.001    |      |
| Intersitial pneumonia vs Bronchopneumonia | 0.003      | 0.538     | 0.447      |      |
| Intersitial pneumonia vs Hilar shadow thickening | 0.001      | 0.420     | 0.442      |      |
| Bronchopneumonia vs Hilar shadow thickening | 0.363      | 0.737     | 0.894      |      |

All values were P values for LSD-t test. CRP = C-reactive protein, D-D = D-dimer, FIB = plasma fibrinogen.

Table 4

Correlations (r value) between CRP, ALT, Anti-MP IgM and blood coagulation indexes.

| Indexes                         | PT (s)   | P    | APTT (s) | P    | FIB (g/L) | P    | TT (s) | P    | D-D (mg/L) | P    |
|---------------------------------|----------|------|----------|------|-----------|------|--------|------|-------------|------|
| CRP (mg/L, in all cases)        | 0.245    | .014 | -0.103   | .305 | 0.355     | <.001| -0.223 | .025 | 0.150       | .134 |
| CRP (mg/L, in all subjects)     | 0.186    | .006 | -0.118   | .080 | 0.390     | <.001| -0.074 | .275 | 0.226       | .001 |
| ALT (U/L, in all cases)         | -0.054   | .595 | -0.203   | .043 | 0.070     | .491 | -0.103 | .307 | 0.602       | <.001|
| ALT (U/L, in all subjects)      | -0.013   | .849 | -0.123   | .069 | -0.020    | .766 | -0.013 | .847 | 0.399       | <.001|
| Anti-MP IgM (U/mL, in all cases)| -0.048   | .634 | -0.221   | .027 | -0.072    | .475 | 0.107  | .288 | -0.001      | .994 |

All subjects refer to all cases with mycoplasma pneumoniae pneumonia and controls; ALT = alanine transaminase, APTT = activated partial thromboplastin time, CRP = C-reactive protein, D-D = D-dimer, FIB = plasma fibrinogen, PT = prothrombin time, TT = thrombin time.
5. Conclusions

Taken together, our results showed that CRP level changed most significantly in group of interstitial pneumonia, whereas FIB, D-D levels changed most significantly in the lung consolidation group. This study may help physicians to estimate the patients' blood coagulation state in time, and give early intervention to prevent severe complications. Further studies conducted in multiple centers with functional assays are needed to validate our findings.

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Author contributions

Juan Wang conceived and designed the idea, did data collection, wrote and drafted the manuscript. Jianping Mao, Gang Chen and Yuanmei Huang did data collection. Jinjin Zhou did literature review. Changlong Gao and Danting Jin reviewed the manuscript. Chenying Zhang performed the data analysis. Juan Wen and Jun Sun designed, contributed to the reviewing of the final manuscript. All authors approved the final format of the submitted manuscript.

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Correction

When originally published, the corresponding author appeared as Juan Wen. This has been corrected to Jun Sun.

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