Elevation of serum alpha-1-acid glycoprotein in children with bronchial pneumonia caused by Mycoplasma pneumoniae infection

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Research article

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Abstract

Background: Alpha-1-acid glycoprotein (AGP) is an acute phase protein which can be elevated under inflammatory conditions. Few studies have explored the use of monitoring serum AGP in Mycoplasma pneumoniae pneumonia (MPP).

Methods: We aimed to evaluate the relationship between serum AGP and other conventional inflammation bio-markers, in order to explore its clinical significance in children with MPP. Pediatric inpatients with bronchopneumonia from January to December 2018 were enrolled and divided into two groups - children with MPP and children without MPP served as controls. Serum AGP, procalcitonin (PCT), and other laboratory examinations were compared and multiple logistic regressions analysis was used to select the main related factors, then receiver-operating characteristic curves were used to evaluate the value of the selected markers used to identify MPP from bronchopneumonia.

Results: Two hundred and sixty-eight children were enrolled in this study. Among them, 87 (32.46%) cases were diagnosed as MPP and 181 gender-matched children were without MPP. Children with MPP were slightly older than those without MPP and had lower levels of serum PCT (P<0.05). In addition, serum AGP was significantly higher in patients with MPP than control participants (136.06±46.37 vs. 122.29±44.21, respectively; P=0.020). Multiple logistic regressions analysis adjusted for age showed that only AGP was the main related factor, which could perhaps be used to identify MPP from common bronchopneumonia and its area under the curve (AUC) was 0.703 (95% CI: 0.634-0.772; P=0.000). Combination with AGP and PCT could slightly improve the AUC (95% CI) to 0.732 (95% CI: 0.666-0.797, P=0.000).

Conclusion: AGP is perhaps a potential bio-marker to distinguish MPP from other bronchopneumonia in children and assist early diagnosis and treatment as soon as possible for it is relatively convenient and fast to be carried out.

Introduction

As a cell-wall-free atypical bacterium, *Mycoplasma pneumoniae* (*M. pneumoniae*) is an important pathogen of pediatric community-acquired pneumonia (CAP)[1]. *M. pneumoniae* has various manifestations dependent on the site of infections, but it most commonly correlates with respiratory tract infection in children and adolescents aged 5 to 14 years old. In recent years, the rate of *M. pneumoniae* infection has shown an obvious upward trend. In hospitalized children, *M. pneumoniae* pneumonia (MPP) can reach 10–40% of CAP[2, 3].

Due to the lack of cell wall and resistance to many common antibiotics, *M. pneumoniae* is resistant to many common antibiotics, thus choices for antimicrobial therapy are limited, and its clinical symptoms are often gradual in onset and nonspecific, making the diagnosis of MPP a major problem in pediatrics. Previous studies have shown that severe or fatal MPP are increasingly occurring[4]. Therefore, rapid laboratory diagnosis of *M. pneumonia* infection is particularly important. It is believed that the
pathogenesis and severity of MPP mainly depends on the direct invasion of lung or bronchial tissues by *M. pneumoniae* and the complex immune response to the infection of the host. It is reported that the host's immune responses is excessive and may play an important role in the development of severe or refractory MPP[5, 6].

Alpha-1-acid glycoprotein (orosomucoid, AGP), largely synthesized by the liver, is a kind of acute phase proteins (APP) which serum concentration can be increased by two to five times under inflammatory conditions[7, 8]. There are few studies on the AGP use in MPP, so in this study we aimed to ascertain the clinical role and value of serum AGP in pediatric MPP and to discover new clues for early diagnosis and therapy of MPP.

**Material And Methods**

**Subjects and data collection**

The study was conducted in children (<16 years) with bronchopneumonia admitted to the pediatric department of our hospital from January to December 2018. They were divided into two groups: children with MPP and children without MPP (NMPP) severed as controls. The diagnosis of MPP was based on the presence of (i) met CAP diagnostic criteria[9] issued by the American Thoracic Society and the Infectious Diseases Society of America in 2007 or has clinical symptoms and signs of pneumonia such as cough, auscultation, and lung infiltration on chest radiographs; and (ii) the antibody titer of anti-mycoplasma was 1:160 or greater, *M. pneumoniae* DNA was positive, or the microorganism was isolated by respiratory specimens culture. Exclusion criteria included patients with (i) recurrent respiratory tract infections, congenital heart diseases, bronchopulmonary dysplasia, neurological disorders, hereditary metabolic diseases, pulmonary embolism, empyema, lung abscess, tuberculosis, and immunodeficiency; or (ii) history use of immunosuppressive agents, immunoglobulin and other drugs; or (iii) co-infected with other pathogens, such as typical bacterial pneumonia, viral pneumonia; or (iv) unknown clinical data. Our study was approved by the hospital's Medical Ethics Review Committee, and informed consent was obtained from a parent or guardian of each participant.

**Clinical assessment and infectious parameters**

Upon admission, patients were subjected to detailed medical examination and demographic data such as name, gender, age and so on were collected. At the day of the study visit, sufficient fasting blood was collected by a forearm vein venipuncture through an aseptic technique and the specimen were immediately sent for laboratory measurements. The peripheral blood in EDTA-K2 anticoagulant tubes was used to detect white blood cell counts (WBC), C-reactive protein (CRP), procalcitonin (PCT), and that in non-anticoagulant tubes was centrifuged and the supernatant serum was collected for other biochemical examinations.
CRP levels were measured via the Ottoman 1000 automatic specific protein instant detection analyzer (Upper Bio-tec Pharma Co., Ltd, Shanghai, China) and procalcitonin levels were assessed via electrochemiluminescence immunoassay on COBAS e601 analyzer (Roche Diagnostics, Basel, Switzerland). Other parameters, such as WBC and levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), total bilirubin (TBil), total protein (TP), albumin (ALB), prealbumin (PA), creatine kinase (CK), creatinine kinase MB isoenzyme (CK-MB), were also conducted via internationally accepted laboratory.

The specific IgM and IgG antibodies against *M. pneumoniae* were analyzed by passive particle agglutination using a commercial diagnostic kit (Fuji Rebio Inc., Tokyo, Japan) according to the manufacturer’s instructions.

Nasopharyngeal aspirates obtained within 24h after hospitalization were added 1 milliliter RNase free normal saline and stored at −80°C. The load of *M. pneumoniae* was measured by quantitative diagnostic kit (ZhiJiang Co., Ltd. Shanghai, China). Real-time fluorescence quantitative PCR was performed using a SLAN-96P detector (Hongshi Co., Ltd., Shanghai, China) and the cycling conditions were as follows: 37 °C for 2 min, 94 °C for 2 min, followed by 40 cycles of 93 °C for 15 sec and 60 °C for 1 min.

**Measurement of AGP**

AGP were analyzed via immunoturbidimetric kits purchased from the Austria Dialab Company on module COBAS c702 analyzer (Roche Diagnostics, Basel, Switzerland). The methodologies had passed the performance verification, and the inspection operation, instrument maintenance, and quality control were strictly carried out in accordance with the requirements of the laboratory operating instructions.

**Statistical methods**

Data were expressed as (mean ±SD) or median (interquartile range (IQR)) for quantitative variables with normally or nonnormally distributed continuous variables as checked via Kolmogorov-Smirnov test, respectively. Comparisons of categorical variables between groups were analyzed by Chi-square test or Fisher’s exact test, while differences of quantitative variables between groups were tested with Student T-test or Wilcoxon test, according to distributions. Multiple logistic regressions were used to evaluate predictors of MPP. The variables that proved significant predictors in logistic regression were studied further using receiver operating characteristic (ROC) curves’ analysis. The area under the curve (AUC) for each parameter was estimated and compared with respect to their diagnostic and prognostic capabilities in patients with MPP. All statistical analysis were performed by the statistical package for social science statistical software version 19.0. A 2-tailed *P* < 0.05 was considered statistically significant.

**Results**
**Patients characteristics**

Totally two hundred and sixty-eight children (139 males and 128 females, with age ranging from 2 months to 14 years) were enrolled in this study. Among them, 87 (32.46%) cases were diagnosed as MPP and 181 gender-matched children without MPP (NMPP) served as controls. The median age of MPP children was 5 years old, and the sex ratio was 0.78 (38:49). Children with MPP were slightly older than those without MPP and had lower levels of serum PCT \((P<0.05)\). In addition, serum AGP concentrations were significantly higher in patients with MPP than in control participants (136.06±46.37 vs. 122.29±44.21, respectively; \(P=0.020\)). There were no significant differences in gender distribution; concentration of serum ALB, PA, CRP; levels of liver function and myocardial enzymes examinations between the two groups \((P>0.05)\) (Table 1).

**Correlation between AGP and other infection parameters**

The correlation between AGP and other inflammatory biomarkers was investigated and the statistical test results (correlation coefficients and \(P\)-values) were listed in Table 2. Mean AGP concentrations were significantly positive associated with CRP levels \((r=0.516, P=0.000)\), PCT levels \((r=0.469, P=0.000)\) and age \((r=0.135, P=0.027)\). While no significant correlation between AGP concentrations, gender distribution, and WBC was observed \((P>0.05)\).

**Diagnostic and prognostic capabilities of selected markers used to identify MPP in bronchopneumonia**

Multiple logistic regressions adjusted for age showed that only AGP was independent related factor for \(M. pneumoniae\) infection. The odds ratio (95% CI) of AGP was 1.009 (1.002-1.015) (Table 3).

In the ROC curves’ analysis, the AUC (95% CI) for AGP and PCT levels as a predictor of MPP prognosis was 0.703 (95% CI: 0.634-0.772; \(P=0.000\)) and 0.706 (95% CI: 0.638-0.773; \(P=0.000\)), respectively, better than that of CRP (0.498 [95% CI: 0.425-0.571; \(P=0.954\)]) (Figure 1).

Moreover, the combination of AGP and PCT could increase AUC (95% CI) to 0.732 (95% CI: 0.666-0.797, \(P=0.000\)), which was slightly superior to any single index (Figure 1).

**Discussion**

\(M. pneumoniae\) infection may cause severe pneumonia and extra-pulmonary which might be life-dangerous though most of \(M. pneumoniae\) infections are mild and self-limited diseases [10–12]. According to statistics, approximately 18% of children with MPP require hospitalization [13]. The rate of \(M. pneumoniae\) infection in pediatric patients with bronchopneumonia was about 32.46% (87/268) in our study, older than that of the control subject (53, 7] years old \(vs.\) 3 ([2, 4] years old), similarly to other previous studies [14–15].
Due to the nonspecific manifestations and lack of reliable diagnostic test to confirm, many MPP are likely to be undetected[16]. It is difficult to make clinical diagnosis of *M. pneumoniae* infection and distinguish MPP from other viral or bacterial pneumonia only based on clinical manifestations, and the drug treatment diagnosis needs a certain time limit, so prompt *M. pneumoniae* testing could be helpful for early detection and treatment of MPP[17–18]. However, *M. pneumoniae* isolation from swab or body fluid specimens needs a long incubation period (10–14 days) and has a low positive rate, which limits the significance of early clinical diagnosis; the development of MP-DNA is not universal and often requires a longer test cycle; and serological diagnosis is due to the low positive rate of MP antibody in the early stage of infection and is related to the immunity of children, and double serological detection execution efficiency is usually general, even if the need for multiple tests, but also need a certain time interval, so its early diagnosis also has greater limitations[19].

Studies has suggested that the host’s immune responses may play an important role in the development of MPP. For example, Xiaowei Wang et al [20] found that Th17 cells may be involved in the *M. pneumoniae* clearance and immuno-pathological damage during the acute infection and persistent infection, respectively. Our study focused on several infectious parameters and acute phase proteins and we were able to demonstrate a significantly higher increase of serum AGP in bronchopneumonia children with *M. pneumoniae* infected as compared with control subjects. AGP, a 41–43 kDa protein belonging to the immunoglobulin family, is an important representative of acute-phase proteins[7]. It is able to bind and transport several endogenous ligands related to inflammation, and able to inhibit IL-6 and TNF-α production and induce CD163 by the TLR4 pathway to involve in anti-inflammatory on monocyte/macrophages[21, 22]. In the case of inflammation, infection, myocardial infarction, and tumors, the concentration of acute phase reactive protein in plasma is often significantly increased (positive phase reactive protein) or decreased (negative phase reactive protein) due to the increase of tissue necrosis and tissue renewal, which might be used to differentiate acute, sub-acute and chronic pathological states. AGP is considered to be a sensitive indicator reflecting inflammatory activity or acute state. Our study showed that as a risk factor of MPP, AGP had a certain value in MPP diagnosis, while the combination of AGP with PCT could slightly improve the efficiency of differential diagnosis.

As to our knowledge, neutrophils are considered to be critical cells and to play an important part in amplifying inflammation and injure the airways, and macrophages not neutrophils play an important role in the clearance of *M. pneumoniae*[6, 23]. And the accumulation of a large number of neutrophils in the lungs is an important pathological feature of MPP. Lung biopsy also shows ulcerated mucosal surfaces, destroyed ciliated epithelium, extensive monocellular infiltration (macrophages, lymphocytes), and neutrophil infiltration[24]. However, the difference of the WBC and neutrophil count between MPP and the control subjects was not statistically significant (*P* > 0.05) in this study. Probably the reason is that the sample used for the study was small and taken only from one geographic area, therefore, further trials with bigger samples from multicenter may be needed.

In our study, although CRP concentrations were significantly positive associated with AGP levels (*r* = 0.516, *P* = 0.000), there was no significant difference in CRP levels between the two groups (*P* > 0.05), consistently
with previous reports[25]. Jeong JE et al[26] observed that compared to those with bronchopneumonia, children affected by segmental/lobar pneumonia showed a significant increase of serum CRP level. We also found that children with bronchopneumonia, whether with or without MPP, seemed to have lower CRP level alike. Due to that CRP is mostly elevated in bacterial pneumonia but only a slight increase in severe viral acute respiratory syndrome, these findings may suggest that *M. pneumoniae* infection is more likely to resemble a viral infection than a bacterial infection[27].

PCT are precursors of thyroid hormone, which has been always used as a biomarker of bacterial infection. Serum PCT level can increase within 2 to 4 hours and peak within 6 to 24 hours of systemic infection, earlier than CRP level[28]. It can often help distinguish a severe bacterial infection from a milder bacterial or viral infection. Our study showed that children with MPP had lower PCT levels (*P* < 0.05), which were also significantly associated with AGP levels (r = 0.469, *P* = 0.000), and were of great value in differentiating MPP from bronchopneumonia with AUC 0.706 (95% CI: 0.638–0.773; *P* = 0.000), similarly to other previous studies[29].

The study has several limitations. First, though all the children were admitted to carry out many traditional microbiological examinations, sputum cultures were not carried out in all proportions to give more solid evidence of bacterial infection. Second, there is no assessment of the severity of the disease and the value of this trial may be low in those with mild MPP although measuring serum AGP levels may help to detect patients with severe MPP. As described above, further prospective trials with bigger samples from multicenter may be needed to confirm the conclusion.

**Conclusions**

The present study focused on clinical and laboratory findings in children with MPP and observed elevated AGP in pediatric MPP. Serum AGP detection is helpful to differentiate MPP from other bronchopneumonia and to assist clinical diagnosis and treatment as soon as possible as it is relatively convenient and fast to be carried out.

**Declarations**

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Not applicable.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Abbreviations

CAP Community-acquired pneumonia
MPP *Mycoplasma pneumoniae* pneumonia
AGP Alpha-1-acid glycoprotein
WBC White blood cell counts
CRP C-reactive protein
PCT Procalcitonin
ALT Alanine aminotransferase
AST Aspartate aminotransferase
GGT Gamma-glutamyl transferase
LDH Lactate dehydrogenase
TBil Total bilirubin
TP Total protein
ALB Albumin
PA Prealbumin
CK Creatine kinase
CK-MB Creatinine kinase MB isoenzyme
ROC Receiver operating characteristic
AUC The area under the curve
IL-6 Interleukin-6
TNF-α Tumor Necrosis Factor-α
Authors’ contributions

HF carried out the main studies and wrote the main manuscript; AZ participated in collecting and analyzing clinical data; SY is to take responsibility for study design. All authors reviewed and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Medical Ethics Review Committee of Shanghai East Hospital affiliated to Tongji University School of Medicine, and informed consent was obtained from a parent or guardian of each participant.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

TABLE 1. Comparison of clinical characteristics of children with MPP and control subjects
### TABLE 2. Correlation analysis between AGP levels and other bio-markers

| Variables | AGP | P-value |
|-----------|-----|---------|
| Age       | 0.135 | 0.027   |
| Gender    | -0.005 | 0.936  |
| WBC       | 0.043 | 0.481   |
| PCT       | 0.469 | 0.000   |
| CRP       | 0.516 | 0.000   |

Abbreviations see Table 1.

### TABLE 3. Odds ratios for MPP and multiple logistic regressions adjusted for age
Abbreviations see Table 1.

**Figures**

![ROC curve](image)

**Figure 1**

Receiver-operating characteristic curves for selected markers used to identify MPP from bronchopneumonia; abbreviations see Table 1