Antibodies against atypical pathogens and respiratory viruses detected by Pneumoslide IgM test in adults with community-acquired pneumonia in Guangzhou City

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Abstract

Background: To detect the serum antibodies against respiratory viruses and atypical pathogens in adults with community-acquired pneumonia (CAP) in Guangzhou City (Guangdong province, China).

Methods: A retrospective study was carried out with samples from 685 adults who were admitted with CAP and 108 non-CAP control patients. Atypical pathogens and respiratory viruses in serum were detected using the Pneumoslide IgM test from Vircell, Spain. All patients were divided into 6 groups according to age: 18-24, 25-44, 45-59, 60-74, 75-89, and >90.

Results: The total positive rate of CAP was 35.4%, which was highest in the 18-24 age group (P < .05). The highest positive rate, 17.11%, was observed for Mycoplasma pneumoniae (MP). The mean age of MP-infected patients was higher than that of the controls (P < .05). The positive rates for influenza B (INFB), Legionella pneumophila (LP1), Coxiella burnetii (COX), influenza A (INFA), parainfluenza virus (PIV), respiratory syncytial virus (RSV), Chlamydophila pneumoniae (CP), and adenovirus (ADV) were 5.56%, 3.07%, 2.63%, 2.34%, 1.90%, 1.61, 0.88%, and 0.29%, respectively. There were 4.37% of patients with CAP having multiple infections. The main symptoms observed in the 685 CAP patients were cough and sputum production, in 78.4% and 67.4%. Fever was followed by 54% of CAP patients. Dyspnea (39.1%), anorexia (36.8%), increased thirst (26.7%), chills (18.7), headache (14.6%), and nausea (13.1%) were also frequently observed in the CAP patients.

Conclusions: MP infection was the most common in adult CAP patients in Guangzhou City with the highest positive rate in the 18-24 age groups.

Keywords: atypical pathogens, community-acquired pneumonia, Guangzhou City, pneumoslide IgM test, respiratory viruses
1  |  INTRODUCTION

Community-acquired pneumonia (CAP) is a common infectious disease worldwide, with a mortality rate of 2%-14%. Recently, it has been found that the importance of atypical bacterial pathogens and viruses in CAP has been underestimated. Previous study has shown that some of CAP were caused by atypical bacterial pathogens and viruses. Jain et al studied 3634 American adults with CAP and found that the most common pathogens were human rhinovirus (in 9% of patients), influenza virus (in 6%), and Streptococcus pneumoniae (in 5%). Chen et al studied 1204 children with pneumonia and found that MP was the most dominant pathogen, followed by influenza B (INFB), parainfluenza virus (PIVs), and respiratory syncytial virus (RSV).

Viruses, as a cause of CAP, is more common in children than in adults. However, the study of its importance in adults is relatively insufficient. The assessments of the prevalence of viral infection in adults with CAP based on a large study population in China have only been performed in Beijing, Shanghai, and Jinan. With the understanding of atypical pathogens, evaluations of their role in lower respiratory tract infections have been gradually increasing. However, reports of viruses and atypical pathogens in adults with CAP remain scarce.

Currently, the greatest challenge in the diagnosis and treatment of CAP in China is the lack of etiological diagnosis in everyday clinical practice. The available methods for etiological diagnosis of infection are serology, culture, and PCR. Pneumoslide IgM, an indirect immunofluorescent assay, allows the identification of IgM antibodies in human serum/plasma against the main etiological agents in respiratory infectious diseases (ie, Legionella pneumophila serogroup 1, Mycoplasma pneumonia (MP), Chlamydia burnetii, Chlamydia pneumoniae, adenovirus, respiratory syncytial virus, influenza A, influenza B, and parainfluenza serotypes 1, 2, and 3). This technique, which has been considered as a useful diagnostic tool for the detection of atypical pneumonia, has been approved by the China Food and Drug Administration (SFDA). It is currently utilized in general hospitals and community hospitals throughout China. To further understand the spread of the atypical pathogens and respiratory virus and the clinical characteristics in adults with CAP in Guangzhou City, a retrospective study of available laboratory and hospital admission data from adults with CAP was performed in the Guangdong Provincial Hospital of Chinese Medicine in Guangzhou.

2  |  PATIENTS AND METHODS

2.1  |  Patients

This study was reviewed and approved by the ethics committee of Guangdong Provincial Hospital of Chinese Medicine. Because this was a retrospective study of medical record data with no patient contact and no collection of personal data, the study was exempt from obtaining informed consent. The adult patients with CAP who were hospitalized in the Guangdong Provincial Hospital of Chinese Medicine between September 2014 and October 2015 were included in this study. Inclusion criteria were as follows: (a) The patient's age is older than 18 years old; (b) patients were identified as showing suspicion of acute respiratory tract infection with a new or progressive infiltrate on a chest radiograph; (c) fever (temperature ≥38°C, or hypothermia (temperature <35°C); and (d) patients with confusion/disorientation and the symptom of an acute respiratory illness, such as a new cough with or without sputum production, abnormal percussion and presence of fine crackles on auscultation, dyspnea, tachypnea, respiratory failure, and leucocytosis or leukopenia.

The unmatched controls (at a rate of 2-3 persons per week) were randomly selected between September 2014 and October 2015 for the comparative purposes to obtain baseline rates. A total of 108 non-CAP control adult patients were admitted to the same hospital for reasons other than respiratory symptoms or acute infection (eg, individuals with muscle sprains).

2.2  |  Sample collection

A total of 3 mL of venous blood were drawn from each patient within 24 hours after admission. The specimens were sent to the clinical laboratory immediately and were centrifuged at 11268 g for 20 minutes at 4°C. The serum was obtained and stored at −20°C until assayed with the Pneumoslide IgM test.

2.3  |  Pneumoslide IgM test (Vircell, Granada, Spain)

A 1:2 dilution of serum samples were prepared with phosphate-buffered saline (PBS) and treated with anti-human IgG sorbent. Then, they were added to every well containing the Pneumoslide IgM slide, respectively. After incubating for 90 minutes at 37°C, the slide was washed twice with PBS and dried. The fluorescent secondary antibody was added and incubated at 37°C for 30 minutes. Then, the slide was washed twice with PBS and observed under the microscope.

Apple green fluorescence was observed in nuclear, cytoplasmic, and/or peripheral in 1%-15% of the cells for positive samples with adenovirus, influenza, VSR, or parainfluenza (peripheral pattern was most frequent with weak samples; in parainfluenza and VSR dyed syncytia together with the previous pattern can be observed). Apple green fluorescence in all the bacteria in the case of Legionella, Chlamydia, or Coxiella can be observed. Apple green fluorescence in the periphery of the cell for positive samples to Mycoplasma can be observed. A negative sample showed no fluorescence for Legionella, Chlamydia, and Coxiella and red cellular pattern for Mycoplasma, adenovirus, influenza A and B, VSR, and parainfluenza. The apple green fluorescent signal was detected using a fluorescence microscope.
Antigens corresponding to each of the following pathogens were present in each slide: LP1, MP, COX, CP, ADV, RSV, INFA, INFB, and PIV 1, 2, and 3.

2.4 | Statistical analysis

Statistical analysis was performed using the SPSS 17.0 software program (SPSS). Quantitative data were presented as the means ± standard deviation (SD). The categorical variables were expressed as frequencies and percentages and were compared using chi-square or Fisher’s exact test. Differences for the pathogen detection rates in the various groups were examined using the chi-square test. The positive rates in different age groups were compared using the chi-square test. Comparisons of the mean age between adults with the three most prominent pathogens and controls were performed by analysis of variance (ANOVA). P < .05 was considered to be statistically significant.

3 | RESULTS

3.1 | Demographics

From September 2014 to October 2015, 685 hospitalized CAP adult patients and 108 non-CAP control patients were included in the study. The demographic characteristics of the CAP patients were shown in Table 1. There was no significant difference in gender and comorbidities (liver disease, cerebrovascular disease, hypertension, and diabetes mellitus) between the two groups. The age of patients with CAP was higher than that of control patients (68.77 ± 18.56 vs 60.9 ± 20.53) (P < .01). The percentage of kidney disease in CAP patients was significantly higher than that in control patients (18.8% vs 10.2%) (P < .05).

| TABLE 1 | Demographics of CAP patients and non-CAP controls |
| --- | --- | --- |
| Characteristics | CAP patients (n = 685) | Controls (n = 108) | P value* |
| Age (means ± SD) | 68.77 ± 18.56 | 60.9 ± 20.53 | <.01** |
| Range (years old) | 16-102 | 16-96 | |
| Gender [n (%)] | | | .49 |
| Male | 382 (55.7%) | 57 (52.3%) | |
| Female | 303 (44.3%) | 52 (47.7%) | .27 |
| Comorbidity [n (%)] | | | |
| Liver disease | 118 (17.2%) | 12 (11.1%) | .103 |
| Kidney disease | 129 (18.8%) | 11 (10.2%) | <.05 |
| Cerebrovascular disease | 90 (13.1%) | 15 (13.9) | .859 |
| Hypertension | 311 (45.4%) | 53 (49.1%) | .531 |
| Diabetes mellitus | 137 (20%) | 24 (22.2%) | .626 |

Note: Comparisons were made using ANOVA for quantitative data and chi-square or Fisher’s exact test for categorical variables.

*P < .05 and **P < .01 vs control.

3.2 | Breakdown of the detected pathogens

The results were shown in Table 2. Among the 685 patients, the total positive rate was 35.4%. A positive rate of 17.11% was observed for MP, which was the highest among the nine examined pathogens. The next most commonly detected pathogen was INFB (5.56%). The detection rates of LP1, COX, INFA, PIVs, RSV, CP, and ADV were 3.07%, 2.63%, 2.34%, 1.90%, 1.61%, 0.88%, and 0.29%, respectively. The positive rate of LP1 in CAP patients was higher than that of controls (3.07% vs 0%). The positive rates of other pathogens were similar between the two groups (P > .05).

3.3 | Mixed infection modes of the pathogens

There were 30 patients in which two or more pathogens were detected, representing 4.37% of the samples, and the modes of mixed infection were complex (Table 3). Among the specimens shown infection with two pathogens, the MP + PIV mixed infection was the most common, with 5 cases. The next mixed infections were MP + INFA, MP + INFB, and MP + LP1, with 3 cases in each. In addition, 5 cases of co-infections with three pathogens were also been observed in Table 3.

3.4 | The positive rates of the pathogens isolated from different age groups

Overall, the positive rates for the 9 pathogens were 86.7% in the (18-24) age group, 37.7% in the (25-44) age group, 33.00% in the (45-59) age group, 38.9% in the (60-74) age group, 30.2% in the (75-89) age group, and 38.2% in the (>90) age group, respectively. The positive rate of viruses and atypical bacterial pathogens was highest in the (18-24) age group (P < .05). The positive rate of the 3 major pathogens, MP,
symptoms of CAP and were significantly more frequent in the CAP group (78.4% and 67.4%, respectively) in comparison of the control group ($P < .01$). A total of 310 patients with CAP had a body temperature more than 38°C, which was significantly different from controls, and 54% (169/310) of them had 0-3 days of fever. In general, the symptoms of dyspnea (39.1%), anorexia (36.8%), thirst (26.7%), chill (18.7), headache (14.6%), and dizziness (13.1%) were also notably more common in the CAP group than those in the control group ($P < .01$, respectively).

The laboratory results, which were obtained from 665 patients with CAP and 97 controls, demonstrated that the higher percentages of lymphocytes (LYM) and monocytes (MONO) had prognostic significance in patients with CAP ($P < .05$). However, there was no statistically significant difference in white blood cell (WBC) counts, neutrophils (NEU%), hemoglobin (HGB) levels, platelet (PLT) counts, or hematocrit (HCT) between patients with CAP and controls based on the blood test ($P > .05$) (Table 5).

### DISCUSSION

Atypical pathogens and viruses constitute an important challenge in the management of patients with lower respiratory tract infection (LRTI) admitted to pediatric intensive care unit (PICU). To optimize the clinical diagnostic, a detection test for respiratory pathogens must be sensitive, fast, low-cost, and capable to detect a wide range of pathogens.

In this study, Pneumoslide IgM test was used to detect approximately 685 patients with CAP. Our results suggested that the positive rate of total atypical pathogen and respiratory virus was 35.4%, which was similar to that of other studies.\textsuperscript{11,25} There were no significant differences observed in the positive rates of a variety of pathogens in the CAP group compared with the control group ($P > .05$). However, there was no statistically significant difference in white blood cell (WBC) counts, neutrophils (NEU%), hemoglobin (HGB) levels, platelet (PLT) counts, or hematocrit (HCT) between patients with CAP and controls based on the blood test ($P > .05$) (Table 5).

### 3.5 Clinical characteristics and blood test values of CAP patients

The patients’ clinical characteristics were summarized in Table 5. The results revealed that cough and expectoration were the most common


| Pathogens | 18-24 (n = 15) | 25-44 (n = 69) | 45-59 (n = 100) | 60-74 (n = 167) | 75-89 (n = 278) | >90 (n = 55) | P value* | CAP patients | Controls | P value† |
|-----------|----------------|----------------|----------------|----------------|----------------|-------------|-----------|-------------|----------|----------|
| MP        | 5 (33.3%)      | 15 (21.7%)     | 14 (14.0%)     | 31 (14.7%)     | 41 (14.7%)     | 11 (20%)    | .28       | 66.40 ± 20.57 | 54.33 ± 27.33 | <.05     |
| INFB      | 3 (20.0%)      | 5 (7.2%)       | 5 (5.0%)       | 10 (6.0%)      | 15 (5.4%)      | 1 (1.8%)    | .4        | 64.71 ± 20.71 | 49.16 ± 20.18 | .093     |
| LP1       | -              | 4 (5.7%)       | 6 (6.0%)       | 3 (1.8%)       | 7 (2.5)        | 1 (1.8%)    | .27       | 62.38 ± 20.28 | -        | -        |
| PIVs      | 2 (13.3%)      | 2 (2.9%)       | 1 (1.0%)       | 4 (2.4%)       | 0 (0.0%)       | 3 (5.5%)    | -         | -           | -        | -        |
| INFA      | -              | -              | -              | 8 (4.8%)       | 5 (1.8)        | 3 (5.5%)    | -         | -           | -        | -        |
| COX       | -              | -              | -              | 4 (4.0%)       | 2 (2.0%)       | 8 (2.9)     | 2 (3.6%)  | -           | -        | -        |
| CP        | -              | -              | -              | 2 (2.0%)       | 2 (1.2%)       | 2 (0.7)     | -         | -           | -        | -        |
| ADV       | 1 (6.7%)       | -              | -              | 2 (2.0%)       | 1 (1.0%)       | 5 (1.8)     | -         | -           | -        | -        |
| RSV       | 2 (13.3%)      | 1 (1.0%)       | 3 (1.8%)       | 5 (1.8)        | -              | -           | -         | -           | -        | -        |
| Total     | 13 (86.7%)     | 26 (37.7%)     | 33 (33.0%)     | 65 (38.9%)     | 84 (30.2%)     | 21 (38.2%)  | <.01      | 68.77 ± 18.56 | 60.9 ± 20.53 | <.01     |

Note: Data in different age group were expressed as n (%). Data in CAP and control group were expressed as means ± SD.

*Comparison of percentage positivity in different age groups by the chi-square test.
†Comparison of the mean age of patients and controls with the three most prominent pathogens by ANOVA.
and we believe that potential mechanisms include immune system dysfunction.

There were also some limitations in this study. There was unequal number of patients in both groups. The detection methods of atypical pathogens and respiratory viruses were signal. More than one detection methods should be performed in further study.

### TABLE 5 Major clinical symptoms and blood test values of the 685 CAP patients

| Symptoms          | CAP patients (n = 685) | Controls (n = 108) | OR (95% CI) | P value* |
|-------------------|-----------------------|--------------------|-------------|----------|
| HT ≥ 38°C         | 310 (45.3%)           | 9 (8.3%)           | 9.18 (4.56, 18.46) | <.01     |
| Days of fever     |                       |                    |             |          |
| 0-1 D             | 80                    | -                  | -           |          |
| 2-3 D             | 89                    | -                  | -           |          |
| 4-5 D             | 65                    | -                  | -           |          |
| 6-10 D            | 51                    | -                  | -           |          |
| 11-15 D           | 9                     | -                  | -           |          |
| >15 D             | 19                    | -                  | -           |          |
| Chills            | 128 (18.7%)           | 6 (5.6%)           | 3.94 (1.69, 9.18) | <.01     |
| Nausea            | 90 (13.1%)            | 26 (24.1%)         | 0.48 (0.29, 0.79) | <.01     |
| Headache          | 100 (14.6%)           | 8 (7.4%)           | 2.15 (1.01, 4.57) | <.05     |
| Myalgia           | 54 (7.9%)             | 5 (4.6%)           | 1.78 (0.69, 4.55) | .22      |
| Fatigue           | 184 (26.7%)           | 21 (19.4%)         | 1.53 (0.92, 2.55) | .09      |
| Rhinorhrea        | 46 (6.7%)             | 4 (3.7%)           | 1.89 (0.66, 5.35) | .29      |
| Nasal discharge   | 51 (7.4%)             | 3 (2.7%)           | 2.84 (0.87, 9.27) | .09      |
| Thirst            | 183 (26.7%)           | 13 (12.0%)         | 2.69 (1.47, 4.92) | <.01     |
| Cough             | 537 (78.4%)           | 25 (23.1%)         | 12.19 (7.52, 19.74) | <.01     |
| Expectoration     | 462 (67.4%)           | 21 (19.4%)         | 8.68 (5.25, 14.34) | <.01     |
| Haemoptysis       | 10 (1.5%)             | 2 (1.9%)           | 0.79 (0.17, 3.66) | .67      |
| Chest pain        | 40 (5.8%)             | 2 (1.9%)           | 3.31 (0.79, 13.93) | .10      |
| Dyspnea           | 268 (39.1%)           | 13 (12.0%)         | 4.74 (2.60, 8.64) | <.01     |
| Vomiting          | 6 (0.9%)              | 2 (1.9%)           | 0.47 (0.09, 2.47) | .30      |
| Anorexia          | 252 (36.8%)           | 23 (21.3%)         | 2.17 (1.33, 3.53) | <.01     |
| Celiacgia         | 23 (3.4%)             | 10 (9.3%)          | 0.34 (0.15, 0.74) | <.01     |
| Blood test        | Value (n = 665)       | Value (n = 97)     |             |          |
| WBCs (×10^9/L)    | 9.38 ± 6.6            | 8.66 ± 5.20        | -           | .299     |
| NEU (%)           | 75.52 ± 30.22         | 71.82 ± 15.00      | -           | .237     |
| LYM (%)           | 19.31 ± 13.38         | 16.34 ± 12.74      | -           | <.05     |
| MONO (%)          | 10.31 ± 31.68         | 7.39 ± 4.96        | -           | <.05     |
| HGB (g/L)         | 112.08 ± 26.62        | 113.66 ± 26.35     | -           | .585     |
| PLT (×10^9/L)     | 194.18 ± 98.53        | 211.72 ± 123.52    | -           | .114     |
| HCT (%)           | 34.04 ± 7.30          | 34.57 ± 7.13       | -           | .501     |

Note: Data in symptoms were expressed as n (%). Data in blood test were expressed as means ± SD.

Abbreviations: CI, confidence interval; HCT, hematocrit; HGB, hemoglobin; HT, highest temperature; LYM, lymphocytes; MONO, monocytes; NEU, neutrophils; OR, odds ratio; PLT, platelets; WBCs, white blood cells.

*ANOVA was used to compare the differences for quantitative data, and chi-square or Fisher's exact test was used to compare the differences for categorical variables.

Based on the clinical characteristics observed in this study, clinicians should remain on high alert for patients who may suffer from CAP if they express the following symptoms: highest body temperature over 38°C within 3 days of onset, cough, expectoration, dyspnea, anorexia, thirst, chill, headache, dizziness, and changes in pulmonary consolidation signs. However, these differences are
clinically insignificant unless combined with the identification of atypical pathogens or respiratory viruses.

5 | CONCLUSIONS

In conclusion, our results suggested that MP was the most common causative pathogen in adult patients with CAP and had the highest positive rate in the (18-24) age groups. Compared with the controls, those with pre-existing kidney disease were at a higher risk of developing subsequent CAP. The patients with CAP characterized by cough, expectoration, and fever exhibited obvious systemic and respiratory symptoms. The MP infection in patients with 18-24 years old should be paid more attention to in further study. Furthermore, the study on the etiology and clinical characteristics of adults with CAP in Guangzhou will not only help to determine the etiology of CAP in this area, but also assist in improving the levels of disease diagnosis, treatment, and management in different age groups.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This study was reviewed and approved by the ethics committee of Guangdong Provincial Hospital of Chinese Medicine. Because this was a retrospective study of medical record data with no patient contact and no collection of personal data, the study was exempt from obtaining informed consent.

CONSENT FOR PUBLICATION

Not applicable.

DATA AVAILABILITY STATEMENT

Not applicable.

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