Short Communication

Do Serological Tests Provide Adequate Rapid Diagnosis of Mycoplasma pneumoniae Infection?

Fang-Ching Liu1,2, Po-Yen Chen3*, Fang-Liang Huang2, Chi-Ren Tsai5, Chun-Yi Lee6 and Chen-Fu Lin7

1Department of Pediatrics, Jen-Ai Hospital, and 2Division of Pediatric Infectious Disease, Department of Pediatrics and 3Microbiology Section of the Medical Laboratory Department, Taichung Veterans General Hospital, Taichung, Taiwan, Republic of China

(Received December 17, 2007. Accepted July 2, 2008)

SUMMARY: This study was designed to evaluate the serologic response to Mycoplasma pneumoniae infection. A total of 589 children ≤18 years (190 in the year 2004; 399 in 2005) and 2,073 adults ≥18 years of age (980 in the year 2004; 1,093 in 2005) with respiratory symptoms undergoing serological testing for M. pneumoniae infection. The tests included passive particle agglutination (PA) and enzyme-linked immunosorbent assay (ELISA). The seropositivity rates of M. pneumoniae infection in the years 2004 and 2005 were 6.9 and 10.1%, respectively. The seropositivity rate was significantly higher in children (29.6% in 2005; 23.7% in 2004) than in adults (2.9% in 2005; 3.7% in 2004) (odds ratio, 8.138 in 2004; 13.923 in 2005; 95% confidence interval, 5.077 - 13.045 in 2004; 9.220 - 21.026 in 2005). Paired sera for the PA test were obtained from 32 of 399 children, and 22 of them demonstrated at least fourfold rises in antibody titer. ELISA had a sensitivity of 77.3% and a specificity of 40.0%; PA had a sensitivity of 9.5% and a specificity of 80%. The ELISA test was superior to the PA test in diagnosing acute M. pneumoniae infection in children. Both tests were significantly more sensitive when they were performed 1 week after the onset of infection.

Mycoplasma pneumoniae is an important cause of respiratory tract infections in children as well as adults. The clinical manifestations of M. pneumoniae infection are quite variable, and range from asymptomatic to mild respiratory symptoms (tracheobronchitis, bronchiolitis) to pneumonia and severe extrapulmonary complications (1). The diagnosis of M. pneumoniae infection is clinically based on serological response. Culturing of the agent is difficult and time-consuming, and polymerase chain reaction (PCR) is relatively expensive (2). The sensitivity of the serological response to M. pneumoniae infection depends on whether the sera are collected early or late after the onset of disease. A second serum sample is usually needed to demonstrate seroconversion or a fourfold titer rise for reliable diagnosis. However, in children, only single serum samples are commonly analyzed because their parents normally do not permit the collection of more than one sample. The aim of the present study was to analyze the relationship between M. pneumoniae antibody response and the onset of infection. The seropositivity of M. pneumoniae infection in children and adults over a period of 2 years was also analyzed.

The study population comprised 589 children ≤18 years (190 in the year 2004; 399 in 2005) and 2,073 adults ≥18 years (980 in the year 2004; 1,093 in 2005) with respiratory tract infections. M. pneumoniae antibodies were tested by the passive particle agglutination (PA) test (Serodia-Myco; Fujirebio Inc, Taipei, Taiwan) and enzyme-linked immunosorbent assay (ELISA) test (SeroMP, Savyon diagnostics Ltd., Ashdod, Israel). According to the manufacturers’ instructions, we defined antibody titers equal to or exceeding 1:160 as determined by the PA test (3,4), or a value above the cutoff of the optimal density as determined by the ELISA test (5) to be indicative of acute M. pneumoniae infection. Paired sera were obtained from 32 of 399 children, and 22 of them demonstrated at least fourfold rises in antibody titer. Based on this result, the sensitivity and specificity of the PA and ELISA tests were analyzed. All results were evaluated by the Pearson chi-square with Yate’s correction. A P value ≤0.01 was defined as statistically significant.

Serologic test results were diagnostic of acute M. pneumoniae infection in 45 children and 36 adults in 2004, and in 118 children and 32 adults in 2005 (Table 1). The seroprevalence rate of M. pneumoniae infection in the year 2005 was on average twofold higher than that in 2004 (10.1 versus 6.9%) (Fig. 1). When we compared the seroprevalence rates between the two age groups, we found that the rate was significantly higher in children aged less than 18 years old than in adults (odds ratio, 8.138 in 2004; 13.923 in 2005; 95% confidence interval, 5.077 - 13.045 in 2004; 9.220 - 21.026 in 2005) (Table 1, Fig. 1). In 2005, acute M. pneumoniae infection was diagnosed in 118 of 399 children, and the antibody responses were analyzed. A linear relationship was found between the number of days since the onset of infection and the levels of mycoplasma IgM (ELISA) antibody (Fig. 2). The value of mycoplasma IgM was significantly above the cutoff value in patients that underwent serological testing ≥7 days after the onset of infection. Figure 3 shows the relationship between mycoplasma antibody titers (PA test) and the number of days since the onset of infection. We found that antibody titers increased with the duration of infection; the titers were highest in those who underwent serological testing ≥7 days after the onset of infection. Of the 80 children who had respiratory symptoms for more than 7 days, 65 (81%) had PA titers ≥1:160. Of the 63 children who had respiratory symptoms for less than 7 days, 18 (29%) had PA titers ≥1:160. The difference was significant (P < 0.001). Of the 32 children from whom
paired sera were obtained, 22 had at least a fourfold rise in antibody titers. Of these 22 children, 17 had positive ELISA results (sensitivity, 77.3%; specificity, 40.0%; positive predictive value, 70.8%) and 2 had PA antibody titers ≥1:160 in their first serum sample (sensitivity, 9.5%; specificity, 80%; positive predictive value, 50.0%).

In this study, the incidence of *M. pneumoniae* infection was higher in children ≤18 years of age (23.7% in 2004 and 29.6% in 2005) than in adults (3.7% in 2004 and 2.9% in 2005). *M. pneumoniae* infection in children was endemic in the year 2005, but the serological response among adult patients from 2004 and 2005 only averaged 3.3%. The seroprevalence of acute *M. pneumoniae* infection in our hospital was lower than that reported in the most recent *M. pneumoniae* study in Taiwan (6). In that study, 6% of healthy adolescents had a current or acute *M. pneumoniae* infection (6). However, the study population was composed primarily of college freshmen with a mean age of 19.7 years, while the mean age of adults in our study was 60.4 years. Younger aged adolescents and children have persistently higher antibody titers for *M. pneumoniae* in PA tests than adults. Mycoplasma antibody levels are usually not detectable or are low in older patients because of reinfection or past infection (7). For accurate diagnosis of *M. pneumonia* infection in adults, paired sera should be combined with PCR detection on respiratory tract specimens (5,8).

Serology tests such as PA and ELISA remain the most practical and convenient methods for laboratory diagnosis of recent *M. pneumoniae* infection; however, the sensitivity of both tests depends on the number of days since disease onset (4-9). Our study showed that the positive predictive value of *M. pneumoniae* IgM is higher for ELISA than for PA in children. The ELISA test is suitable for serological diagnosis of *M. pneumoniae* infection in children using unpaired serum taken during the early stage of infection (9). The IgM-capture ELISA test is used to detect *M. pneumoniae*-specific IgG, IgM, and IgA antibodies (7), while the PA test is only indicative of IgM-class antibodies (10). The sensitivity of the PA test depends on the number of days since disease onset, and the IgM antibody is usually absent in the course of early infection (8,9). For example, in patients from whom serum samples were collected during the early stage of infection (<7 days since onset), only 29% showed PA antibody titers ≥1:160; however, in patients from whom serum samples were collected on the 7th day or later since the onset of disease, 81% had titers ≥1:160. This absence of IgM antibody in the early stages of infections explains why the sensitivity of the PA results was low in our study. Because antibody titers in the PA test usually peak 1-3 weeks after the onset of infection, paired serum specimens showing a rise in IgG antibody titers are clinically useful for diagnosing *M. pneumoniae* infection (3,10). In summary, the sensitivity of the IgM ELISA test is higher than that of the PA test in diagnosing acute disease stage. Furthermore, the IgM ELISA test is suitable for early accurate diagnosis of *M. pneumoniae* infection in children using unpaired serum. PA serology shows a significant titer rise when sera are collected ≥7 days after the onset of infection.

**ACKNOWLEDGMENTS**

The work was assisted by biostatistics task force of Taichung Veterans General hospital.
REFERENCES


