Analysis of Complement Fixation and Commercial Enzyme Immunoassays for Detection of Antibodies to *Mycoplasma pneumoniae* in Human Serum

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Received 23 December 1999/Returned for modification 5 April 2000/Accepted 14 June 2000

The Meridian ImmunoCard (IC), GenBio ImmunoWELL-IgM, and Remel EIA commercial antibody tests are qualitative enzyme immunoassays that detect antibodies to *Mycoplasma pneumoniae* in serum. These tests were compared to an *M. pneumoniae* complement fixation (CF) assay, which uses a commercially available antigen component. The Meridian IC and the ImmunoWELL-IgM detect immunoglobulin M (IgM) only; the Remel EIA and the CF test detect both IgM and IgG antibodies. Detection of specific IgM antibody, which appears early in infection, can be, but is not always, indicative of a recent or current infection. Paired serum samples from 64 adult patients with probable *M. pneumoniae* infection were examined with each of the four tests. Thirty (47%) of the 64 acute-phase sera were IgM positive by Meridian IC, 26 (41%) were positive by Remel EIA, 24 (38%) were positive by CF, and 15 (23%) were positive by ImmunoWELL-IgM. When both the acute- and convalescent-phase serum samples from each patient were examined, 61 (95%) of the 64 patients were positive by CF, 60 patients (94%) were positive by Remel EIA, 52 patients (81%) were IgM positive by the Meridian IC, and 29 patients (45%) were IgM positive by the ImmunoWELL-IgM assay. The Meridian IC was more sensitive than the other tests for early detection of IgM antibodies. However, after examining paired serum samples, we concluded that the detection of IgM alone may not be useful for all cases of mycoplasma infection, especially in an adult population.

*Mycoplasma pneumoniae* is an important cause of upper and lower respiratory tract infections, including pharyngitis, tracheobronchitis, and pneumonia, in children and adults of all ages (3, 12). Laboratory diagnosis of *M. pneumoniae* infection has relied mainly on serologic tests because the organism is hard to isolate (5, 6, 9, 11, 18). A reliable and sensitive serologic test for use in the early phase of infection by *M. pneumoniae* is needed to confirm the infection and to ensure that the appropriate antibiotic is used for treatment. The detection of specific immunoglobulin M (IgM) antibody, which appears 7 to 10 days after infection and approximately 2 weeks before IgG antibody, has been shown to indicate a recent or current infection with *M. pneumoniae* (10, 14–16). However, the presence of IgM in adult serum does not always indicate a current infection, because in some cases IgM has been shown to persist for up to a year after infection. In addition, the IgM response is either minimal or undetectable in some cases of adult reinfection with *M. pneumoniae* (5, 10, 16, 19). Therefore, reliance on the detection of specific IgM alone, especially in an adult population, could allow some infections to be missed. In a previous study (19), approximately 20% of adults did not mount an IgM response after infection with *M. pneumoniae*.

We tested paired serum specimens, obtained from 64 adult patients with probable *M. pneumoniae* respiratory infections, with three commercial enzyme immunoassays (EIAs): the ImmunoCard (IC) mycoplasma EIA (Meridian Diagnostics, Cincinnati, Ohio), the Remel EIA *M. pneumoniae* IgG-IgM antibody test system (Remel, Lenexa, Kans.), and the ImmunoWELL-IgM EIA (GenBio, San Diego, Calif.), now marketed through Alexon-Trend, Ramsey, Minn. The paired samples were also tested with a complement fixation (CF) assay, considered to be the serologic “gold standard,” to determine if a more timely diagnosis of *M. pneumoniae* could be obtained in the early phase of infection.

MATERIALS AND METHODS

Sera. Acute- and convalescent-phase sera were obtained from 64 patients during suspected outbreaks of respiratory infections caused by *M. pneumoniae* (4, 13). Most of the patients had chest X rays with infiltrates compatible with atypical pneumonia. Other features of the infections included cough, fever, and myalgias. Sera were held at −20°C before being tested by the Meridian IC, ImmunoWELL-IgM, Remel EIA, and CF tests. Twenty-one paired serum samples from an outbreak of respiratory illness due to parainfluenza virus were also tested with each of the assays. None of the serum samples were linked to individual patient identifiers.

Meridian IC. The Meridian IC mycoplasma EIA is a qualitative procedure for the detection of IgM antibodies to *M. pneumoniae* in human serum. The test was performed according to the manufacturer’s instructions. Briefly, the test system consists of a plastic card with four openings that provide access to absorbent filter paper. The filter paper is impregnated with an *M. pneumoniae* antigen extract in the top right port (test well). The top left port (control well) contains a human IgM reagent impregnated onto the paper. A patient’s serum was added to both lower wells (sample ports) and allowed to migrate to the upper (control and test) wells. Next, an anti-human IgM-alkaline phosphatase conjugate was added to both sample ports and allowed to migrate to the upper ports for 2 min. The upper ports were then washed with buffer. Next, substrate solution was added to the control and test ports and allowed to react for 5 min. The development of a blue color in the test well indicated a positive test result for IgM to *M. pneumoniae*. A blue color in the control well indicated that the test was performed properly.

ImmunoWELL-IgM. The ImmunoWELL-IgM *M. pneumoniae* antibody test, marketed by Alexon-Trend, is a qualitative EIA for the detection of specific antibodies to *M. pneumoniae* in human serum. The test was performed according to the manufacturer’s instructions. Briefly, a patient’s serum that was pretreated with anti-human IgG was added to microtiter wells coated with a purified glycolipid extract of *M. pneumoniae* strain FH and allowed to react for 60 min. After removal of unbound antibodies, horseradish peroxidase-conjugated anti-human antibodies were allowed to react with the bound antibodies for 30 min. Unbound conjugate was removed and a chromogenic substrate was added to each well and allowed to react. After 30 min, stop solution was added and the optical density of each well was read at 405 nm. Optical density readings were normalized before clinical interpretations were made. Values that were <0.77 were reported as...
The CF test, 60 pairs (94%) were positive by the Remel EIA. Of the 61 phase sera were tested, 61 pairs (95%) were positive by the four test methods when both the acute- and convalescent-phase sera were tested. IgM antibodies to M. pneumoniae are more sensitive than the CF test for the early detection of infection. Of the 40 patients that had acute-phase sera with CF titers of <8, 26 (41%) were Remel EIA positive, 24 (38%) were Meridian IC positive, and 15 (23%) were ImmunoWELL-IgM positive. A fourfold rise in antibody titer to a titer of ≥32 or a high standing titer of ≥64 was considered to be evidence of actual infection.

RESULTS

Results obtained from testing only the acute-phase sera from the 64 patients showed that 30 (47%) were Meridian IC positive, 26 (41%) were Remel EIA positive, 24 (38%) were CF positive, and 15 (23%) were ImmunoWELL-IgM positive. Of the 40 patients that had acute-phase sera with CF titers of <64, the Meridian IC was positive for 13, the Remel EIA was positive for 5, and the ImmunoWELL-IgM was positive for 2 (Table 1). These results suggest that in some cases these tests are more sensitive than the CF test for the early detection of IgM antibodies to M. pneumoniae.

All 64 paired serum samples were positive by at least one of the four test methods when both the acute- and convalescent-phase sera were tested. Sixty-one pairs (95%) were positive by the CF test, 60 pairs (94%) were positive by the Remel EIA, 52 pairs (81%) were positive by the Meridian IC test, and 29 pairs (45%) were ImmunoWELL-IgM positive. Comparison of the Meridian IC test with the CF test showed 12 paired sera that were CF positive but Meridian IC negative (Table 2). Ten of these 12 paired sera were also positive by the Remel EIA. Three paired sera were Meridian IC positive and CF negative. Also, two paired sera were Meridian IC positive and Remel EIA negative.

Comparison of the ImmunoWELL-IgM results with the Remel EIA and CF test results showed that the Remel EIA was positive with sera from 31 patients who were ImmunoWELL-IgM negative (Table 3). In addition, the CF test was positive with sera from 32 patients who were ImmunoWELL-IgM negative.

To verify the presence of antibodies to M. pneumoniae, a convalescent-phase serum sample was necessary for 37 (61%) of 61 CF-positive cases, 35 (58%) of 60 Remel EIA-positive cases, 14 (48%) of 29 ImmunoWELL-IgM-positive cases, and 22 (42%) of 52 Meridian IC-positive cases.

DISCUSSION

Alexander et al. (1) found the Meridian IC to be a valid method for detecting IgM antibodies to M. pneumoniae by using acute-phase sera from patients with suspected M. pneumoniae infections. The approach of testing for IgM alone has a theoretical advantage, because only one specimen, collected

| TABLE 1. Number of acute-phase sera which gave negative or positive results by commercial EIAs compared to known CF titers |
|-----------------------------------------------|
| Test result | No. of sera with indicated CF titer* |
|-----------------------------------------------|
| Remel EIA positive | ≤8 | 8 | 16 | 32 | 64 | ≥128 |
| Remel EIA negative | 0 | 1 | 1 | 3 | 6 | 15 |
| Meridian IC positive | 5 | 5 | 10 | 14 | 4 | 0 |
| Meridian IC negative | 5 | 6 | 8 | 8 | 4 | 3 |
| ImmunoWELL-IgM positive | 0 | 0 | 1 | 1 | 4 | 9 |
| ImmunoWELL-IgM negative | 5 | 6 | 13 | 14 | 6 | 5 |

* The titer was defined as the reciprocal of the highest dilution of serum showing 0 to 30% hemolysis. A titer of ≥64 was considered positive.

| TABLE 2. Results of the Meridian IC IgM assay compared to results of the Remel EIA and CF assay |
|-----------------------------------------------|
| Meridian IC result | Remel EIA | CF |
|-----------------------------------------------|
| | No. (%) positive | No. (%) negative | No. (%) positive | No. (%) negative |
|-----------------------------------------------|
| Positive | 50 (78) | 2 (3) | 49 (77) | 3 (5) |
| Negative | 10 (16) | 2 (3) | 12 (19) | 0 (0) |

* A single serum dilution was tested. A visible change in the membrane color in the test well to purple was considered positive.

* A standing titer of ≥64 or a fourfold rise in titer to ≥32 was considered positive.

* A single serum dilution was tested. Normalized optical density readings of ≥770 were considered positive.

| TABLE 3. Results of the ImmunoWELL-IgM assay compared to results of the Remel EIA and CF assay |
|-----------------------------------------------|
| ImmunoWELL-IgM result | Remel EIA | CF |
|-----------------------------------------------|
| | No. (%) positive | No. (%) negative | No. (%) positive | No. (%) negative |
|-----------------------------------------------|
| Positive | 29 (45) | 0 (0) | 29 (45) | 0 (0) |
| Negative | 31 (48) | 4 (6) | 32 (50) | 3 (5) |

* A single serum dilution was tested. A visible change in the membrane color in the test well to purple was considered positive.

* A standing titer of ≥64 or a fourfold rise in titer to ≥32 was considered positive.

* A single serum dilution was tested. Normalized optical density readings of ≥770 were considered positive.
at least 7 to 10 days after the onset of infection, is required. However, a major limitation of an IgM-specific test is that detectable levels of IgM antibodies may not be present if the serum sample is obtained too early (7). Depending on the assay used, this was the case in 42 to 61% of the patients in the present study, from whom a second or convalescent-phase serum specimen was necessary to confirm infection with *M. pneumoniae*.

The Meridian IC and ImmunoWELL-IgM tests, which are specific for IgM, were positive with acute-phase sera from 47 and 23%, respectively, of the adult patients with suspected *M. pneumoniae* respiratory infections. The Remel EIA and CF tests, which detect IgM and IgG simultaneously, were positive with acute-phase sera from 39 and 38%, respectively, of the patients tested. Based on the results of this study, the Meridian IC is the test of choice, especially for children, if only acute-phase sera are available for testing. Convalescent-phase sera are difficult to obtain in many cases; however, all three EIA phase sera are available for testing. Convalescent-phase sera patients tested. Based on the results of this study, the Meridian and 23%, respectively, of the adult patients with suspected *M. pneumoniae* used, this was the case in 42 to 61% of the patients in the serum sample is obtained too early (7). Depending on the assay however, a major limitation of an IgM-specific test is that at least 7 to 10 days after the onset of infection, is required. The CF and Remel EIA, which detects IgM antibodies to *M. pneumoniae* in adults and in cases of reinfection, where IgM is not always produced (5, 9, 15, 16, 19). Fifty-two of 64 patients (81%) were found to have IgM antibodies when paired sera were tested with the Meridian IC test. This is in line with other studies (8, 12), which showed that patients >20 years of age produce IgM antibodies 75 to 82% of the time when infected with *M. pneumoniae*.

The CF and the ImmunoWELL-IgM assays are designed to detect antibodies to glycolipids. The immune response in adults to glycolipids may be prolonged, meaning that a positive IgM test result may indicate a previous infection (10, 16). In addition, adults may produce only IgG antibodies, particularly to protein antigens, which are used in some commercial serology tests (17). Each approach has its advantages and limitations, but caution should be used in interpreting IgM results when using serum from adults >20 years of age.

The combined detection of IgM and IgG antibodies and the use of paired sera increased the accuracy of detection of *M. pneumoniae* infections. The CF and Remel EIA, which detect both IgM and IgG, were positive in 95 and 94% of the cases, respectively. *M. pneumoniae*-specific IgM was not detected with the Meridian IC in 12 cases that were considered positive by CF. Of these 12 cases that were CF positive but Meridian IC negative, eight showed a fourfold rise in CF titer. Five of the eight cases also showed a conversion from negative with the acute-phase sera to positive with the convalescent-phase sera with the Remel EIA, which suggests the presence of an acute infection.

Both the Meridian IC and the Remel EIA tests are rapid to perform and do not require any specialized or expensive equipment. All necessary controls are included with each test kit, and both tests would be cost-effective when performed on a single serum sample or small batches of sera. In addition, because the results are available within minutes with the Meridian IC and Remel EIA kits, these tests may provide a means to rapidly diagnose and treat patients with *M. pneumoniae* infections. However, as shown in this study and previous studies (6, 17, 19), adult serum samples obtained early in the acute phase of *M. pneumoniae* infection may not contain detectable levels of IgM antibody. Ideally, a second serum sample should be obtained after 7 to 14 days if *M. pneumoniae* infection is still suspected.

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