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Molecular detection of *Mycoplasma pneumoniae* in adults with community-acquired pneumonia requiring hospitalisation

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ABSTRACT

Among 144 patients (93 males, mean age 63 years) hospitalised because of community-acquired pneumonia, an aetiologic agent was diagnosed in 93 (65%) patients. *M. pneumoniae* infection was diagnosed in 18 (19%) of them. The infection was demonstrated by PCR in 15 patients and by serology in 10 patients. The mean age of the 8 patients with positive *M. pneumoniae* PCR and negative serology, was significantly higher than that of the 10 patients with positive serology.
INTRODUCTION

The finding of aetiologic pathogens causing community-acquired pneumonia (CAP) depends largely on the laboratory techniques used and the specimens provided to perform the laboratory tests. For a difficult cultivable pathogen like *M. pneumoniae*, which is estimated to be the causative agent in 5% of the patients hospitalised with CAP (1), diagnosis relies mainly on antibody detection in paired serum samples. Rapid diagnosis of *M. pneumoniae* infection however, is essential for the choice of antibiotic regimens to be used in patients with CAP.

Recently, PCR on respiratory specimens has been applied for diagnosis (2-4), but thus far it is unclear which respiratory specimen is most suitable for detection of *M. pneumoniae* DNA in patients with CAP. Therefore, a prospective study among adults hospitalised with CAP was designed (5). In this study patients were extensively sampled from various sites of the respiratory tract. These specimens were processed for *M. pneumoniae* PCR. The results were compared with those obtained by serological testing of an acute and convalescent phase serum sample from each patient.

METHODS

For a 21 month period, September 1992 through June 1994, 144 adults (≥ 18 years) admitted with CAP, to the St Antonius Hospital, Nieuwegein, the Netherlands, were included in the study. CAP was defined as respiratory infection with new or progressive radiographic changes. Upon enrolment, nasopharyngeal and throat specimens were collected using sterile cotton tipped aluminium shafted swabs, which were suspended in 1.5 ml 2SP transport medium. Throat wash samples were obtained using 10 ml phosphate buffered saline. Sputum, bloodcultures, broncho-alveolar lavages, bronchial aspirates and pleural fluid were obtained if possible, using standard procedures. A first serum sample was collected within 24 hours of enrolment, and a second sample at least 10 days thereafter.

**PCR for M pneumoniae.** Two hundred μl of nasopharyngeal and throat specimen or 1.0 ml throat wash sample, bronchial aspirate or broncho-alveolar lavage were transferred to a sterile tube and centrifuged for 30 min at 15,000 x g. Sputum samples were suspended in 1.5 ml 2SP transport medium. 100 μl suspended sample was transferred to a sterile tube and centrifuged. Pellets were subjected to DNA extraction according to Boom et al. (6). DNA extracts were stored at -70 °C until processed for PCR. Ten μl of the extracted DNA was used as template in a nested protocol with P1 gene specific primers (7). An amplification control was added to each reaction tube and in case of inhibition, a dilution of the DNA-extract (1:10 in sterile water) was retested.
**Serology for M. pneumoniae.** Paired sera were analysed by complement fixation test (CFT) using a commercially available *M. pneumoniae* antigen (Virion, Rüschlikon, Switzerland). A fourfold rise in titer or a single titer of ≥ 1:128 was regarded as positive. *M. pneumoniae* specific antibodies were determined with a microparticle agglutination (MAG) test (Serodia-MycoII kit; Fujirebio, Tokyo, Japan). An antibody titer of ≥ 1: 160 was regarded as positive.

**Routine microbiological procedures.** These procedures included blood culture, Gram stain and culture of sputum, culture of pleural fluid. CFT was performed for influenza A and B virus, parainfluenza viruses, respiratory syncytial virus, adeno virus and *Coxiella burnetii*. For *Legionella pneumophila* an indirect immunofluorescent test (IgG and IgM) was applied. For *Chlamydia pneumoniae* three serological tests were performed. Additionally, respiratory specimens were cultured for *C. pneumoniae* and processed for *C. pneumoniae* PCR (5).

**Clinical data.** From all patients clinical data were collected using a standard questionnaire.

**Statistics.** For comparison of the median age of seropositive- and seronegative patients with *M. pneumoniae* infection, the Mann-Whitney U-test was used.

**RESULTS AND DISCUSSION**

During the 21 month period, 144 patients (93 males, 65%) with CAP were included in the study. The mean age was 63 ± 15 years. Underlying disease like chronic obstructive pulmonary disease (COPD) was present in 77 (54%) patients, 4 patients had a malignancy and 6 were immunocompromised. Fifty-nine (41%) patients had used antibiotics prior to enrolment, 38 (65%) β lactam antibiotics, 12 (20%) macrolides or doxycycline, and 9 (15%) other antibiotics.

In total 552 specimens were subjected to *M. pneumoniae* PCR, 144 nasopharyngeal specimens, 144 throat specimens, 139 throat washes, 101 sputum specimens, 11 bronchial aspirates and 13 broncho-alveolar lavages. From each patient two serum samples were obtained. PCR was inhibited in 57 (9.6%) of the 552 specimens tested, with the highest percentage of inhibition in sputum specimens (15%). After diluting these samples, inhibition was resolved in all cases. *M. pneumoniae* DNA was detected in 10% of the sputum specimens, in 5% of the throat washes and nasopharyngeal specimens, and in 3.5% of the throat swab specimens (Table).

Aetiological agents were demonstrated in 93 (65%) patients. The most common pathogens were *Streptococcus pneumoniae* (n=21), *Haemophilus influenzae* (n=22), *C. pneumoniae* (n=23) and influenza A virus (n=9), either alone or in combination. A current or recent *M. pneumoniae* infection was diagnosed in 18 (12.5%) patients (Table).
### Table. Clinical and laboratory findings in 18 adults hospitalised with CAP who were positive for *M. pneumoniae* in any of the laboratory tests applied for diagnosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>day of sampling&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antibiotics used before enrolment</th>
<th>Age</th>
<th>Underlying pulmonary disease</th>
<th>Leukocyte count&lt;sup&gt;b&lt;/sup&gt; (10&lt;sup&gt;9&lt;/sup&gt;/L)</th>
<th>CFT titer&lt;sup&gt;b&lt;/sup&gt; in nasal wash sample</th>
<th>MAG titer&lt;sup&gt;b&lt;/sup&gt; in nasal wash sample</th>
<th>Result of PCR on:</th>
<th>Concomitant pathogens</th>
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<td>1</td>
<td>10</td>
<td>amoxi</td>
<td>33</td>
<td></td>
<td>12.2</td>
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<td>2;128</td>
<td>+</td>
<td>+</td>
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<td>2;128</td>
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<td>7</td>
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<td></td>
<td>12.9</td>
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<td>8</td>
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<td>COPD&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>33.5</td>
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<tr>
<td>18</td>
<td>12</td>
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<td>10.1</td>
<td>4</td>
<td>4</td>
<td>&lt;40</td>
<td>&lt;40</td>
</tr>
</tbody>
</table>

<sup>a</sup> Relative to day of first clinical symptoms
<sup>b</sup> COPD: chronic obstructive pulmonary disease
<sup>c</sup> CFT positivity was defined as ≥128 or a fourfold titer rise
<sup>d</sup> MAG positivity was defined as ≥160

NT: not tested, NA: not available

amoxi=amoxicillin, amox/clav=amoxicillin/clavulanate, peni=penicillin, cephalo=cephalosporin, erythro=erythromycin, cipro=ciprofloxacin, doxy=doxycycline.
Among them, *M. pneumoniae* infection was demonstrated by serological testing only in 3 (17%) patients, by PCR only in 8 (44%) patients, and by both methods in 7 (39%) patients. In 6 of these 7 patients, 2 or more respiratory samples were *M. pneumoniae* DNA positive indicating a high load of *M. pneumoniae*. In 9 patients *M. pneumoniae* DNA was detected in one respiratory sample only. This indicates lower loads of *M. pneumoniae* in the respiratory tract, which can be due to persistence of the bacterium after infection. Among these 9 patients, 6 (67%) had COPD which may be a predisposing factor for persistence (8). Other possible explanations for the difference between positive PCR results and negative serological results can be (i) false positive PCR results; this seems unlikely as all possible precautions to avoid contamination had been taken (7); (ii) lack of immune response; none of the 8 patients with positive PCR and negative serology had a compromised immune system, but their median age (73.5 years) was significantly higher than that of the patients with positive *M. pneumoniae* serology (44.5 years)\(P=0.004\). Impaired immune response in the elderly patients may be a reason for discrepancy between serological and PCR results. In a recent seroepidemiological study of *M. pneumoniae*, significantly lower antibody titers at older age have been demonstrated (9).

In the 3 patients with positive serological tests only, discrepancy between PCR and serological results might be due to a *M. pneumoniae* load below the detection level of the PCR or the absence of *M. pneumoniae* in the two samples tested (patient 10 in the Table). The negative PCR results could not be due to antibiotic treatment, as two patients were not treated with antibiotics before enrolment and one patient received antibiotics only 24 hours before enrolment.

For rapid diagnosis of *M. pneumoniae* infection the MAG test, which was positive in 4 patients, had no additional value. Assays detecting IgM antibodies, like the MAG test (10), have higher sensitivities when used in children with respiratory infection (2, 4).

At least one other pathogen in addition to *M. pneumoniae* was detected in 9 (50%) patients. *C. pneumoniae* has been reported as a common cause of mixed infections in CAP (5, 11), which is in agreement with our finding of 3 patients with *C. pneumoniae* infection concomitant with *M. pneumoniae*. In addition, Lieberman et al. (12) reported identification of at least one other pathogen additional to *M. pneumoniae* in 64% of 101 patients hospitalised for CAP. Like in our study, *S. pneumoniae* and *C. pneumoniae* were the most frequently diagnosed concomitant pathogens.

In conclusion, for rapid diagnosis of *M. pneumoniae* infection in adults hospitalised with CAP, PCR on sputum has the highest yield. Despite use of our *M. pneumoniae* PCR, antibody detection by CFT in an acute and convalescent phase serum remains necessary to increase sensitivity of laboratory diagnosis. Elderly patients with a respiratory sample positive for *M. pneumoniae* DNA and without positive serology, might be deficient in antibody response and should be subjected to further study to clarify the role of *M. pneumoniae* in this patient group.
REFERENCES

1 Foy HM. Infections caused by Mycoplasma pneumoniae and possible carrier state in different populations of patients. Clin Infect Dis 1993; 17 Suppl 1:S37-S46


