

# Detection of *Mycoplasma pneumoniae* in adult community-acquired pneumonia by PCR and serology

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Diagnosis of pneumonia caused by *Mycoplasma pneumoniae* in adults is hampered by a lack of rapid and standardized tests for detection. This prospective study was conducted to compare the diagnostic values of an indirect immunofluorescence assay and a 16S rRNA gene PCR for the diagnosis of *M. pneumoniae* pneumonia in adults. From February 2005 to January 2008, 357 patients (53.8 % males, median age 63 years, range 18–94) admitted for community-acquired pneumonia (CAP) to two hospitals in Santiago, Chile, were enrolled in the study. Thirty-two patients (9.0 %) met the criteria of current or recent *M. pneumoniae* infection, and laboratory diagnosis was definitive in 26 cases (81.2 %) and presumptive in six cases (18.8 %). Among the 32 *M. pneumoniae* infections, the PCR assay was positive in 23 (71.9 %) and the serology in 27 (84.4 %) of the cases. IgM was positive in acute-phase serum specimens in 13 cases (40.6 %) of *M. pneumoniae* infections. Using serology as the gold standard, the sensitivity, specificity, and positive and negative predictive values of the PCR were 66.7, 98.5, 78.3 and 97.3 %, respectively, whereas the global agreement of the methods was 343/357 (96.1 %). The frequency of *M. pneumoniae* CAP cases declined significantly during the second year of study, suggesting the end of an epidemic period. In conclusion, although good global agreement was found between PCR and serology, the lower sensitivity of the PCR leads us to recommend the use of both procedures in parallel to confirm *M. pneumoniae* in CAP in adults.

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**Abbreviations:** CAP, community-acquired pneumonia; IFA, immunofluorescence assay.



## INTRODUCTION

Community-acquired pneumonia (CAP) is a relevant worldwide cause of morbidity and mortality. In spite of technological advances in microbial diagnosis, the aetiology usually remains uncertain and the therapy is empirical, based on the clinical and epidemiological features ([Niederman \*et al.\*, 1993](#); [Fine \*et al.\*, 1997](#)).

*Mycoplasma pneumoniae* is a common cause of CAP, mainly in children and young adults ([Foy, 1993](#); [Waites & Talkington, 2004](#)). During epidemic periods, it also accounts for a significant proportion of acute respiratory tract infections in all age groups ([Rastawicki \*et al.\*, 1998](#); [Layani-Milon \*et al.\*, 1999](#)). Furthermore, around 25 % of people infected with *M. pneumoniae* may experience extrapulmonary complications ([Waites & Talkington, 2004](#)). *M. pneumoniae* infections cannot be distinguished clinically from infections caused by viral or other bacterial agents ([Foy, 1993](#); [Waites & Talkington, 2004](#)). Thus a rapid and accurate laboratory diagnosis is relevant for an effective therapy and for limiting the spread of infection in the community. The diagnosis of *M. pneumoniae* infection is currently based principally on serology. The detection of IgM provides an early and sensitive diagnosis in children ([Waris \*et al.\*, 1998](#); [Petitjean \*et al.\*, 2002](#)). However, several studies have shown that adults fail to respond with IgM, probably as a result of re-infections ([Sillis, 1990](#); [Uldum \*et al.\*, 1992](#); [Jacobs, 1993](#)). As IgG during the acute infection may represent persistent antibodies from past infections, serological diagnosis relies on serology of paired sera, although its use is limited by the delay in getting the convalescent sample ([Jacobs \*et al.\*, 1986](#); [Sillis, 1990](#); [Csángó \*et al.\*, 2004](#); [Beersma \*et al.\*, 2005](#); [Nir-Paz \*et al.\*, 2006](#)). A variety of commercial immunodiagnostic tests, such as ELISA and immunofluorescence, which need appropriate validation, are now available for serology. In addition, nucleic acid amplification methods such as PCR have become an attractive diagnostic alternative for acute *M. pneumoniae* infections, but much work is needed until they can be adopted as routine procedures in public settings as well as in clinical laboratories ([Tjhie \*et al.\*, 1994](#); [Falguera \*et al.\*, 1996](#); [Dorigo-Zetsma \*et al.\*, 2001](#); [Loens \*et al.\*, 2003](#); [Templeton \*et al.\*, 2003](#); [Waites & Talkington, 2004](#)).

The aim of this study was to compare an indirect immunofluorescence assay (IFA) for detection of IgM and IgG antibodies with a PCR assay for the diagnosis of *M. pneumoniae* in adults presenting with CAP in samples collected over a 3-year period in Santiago, Chile.

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## METHODS

**Study population.** A prospective study was conducted in patients  $\geq 18$  years of age with CAP, which was radiographically confirmed, in two hospitals (Hospital Clínico Universidad de Chile and Hospital de Infecciosos Dr Lucio Córdova) in Santiago, Chile, between February 2005 and January 2008. Patients with immunosuppression and those who had received immunosuppressive therapy or had been hospitalized in the previous 30 days were excluded. The study was approved by the university and the health institutional review board and all patients gave their informed consent to participate.

**Microbiological laboratory tests.** Throat washes and first serum samples were obtained on admission, and convalescent serum samples were obtained 4–6 weeks later. Throat washes were performed with 5 ml Hanks' balanced salt solution and immediately transported on ice to the laboratory; specimens were stored at  $-20^{\circ}\text{C}$  until PCR testing, usually within 3 days. Serum specimens were stored at  $-20^{\circ}\text{C}$  until processed.

For the detection of *M. pneumoniae*, a 277 bp segment of the 16S rRNA gene was amplified by PCR as described by [Tjhie et al. \(1994\)](#). For PCR, aliquots (250  $\mu\text{l}$ ) of each specimen were extracted using a QIAmp DNA Mini kit (Qiagen) according to the manufacturer's instructions. The extracted DNA was eluted in 70  $\mu\text{l}$   $\text{H}_2\text{O}$  and immediately used in the amplification reaction. Positive (*M. pneumoniae*, strain FH) and negative (sterile water) controls were included in each PCR run. To rule out PCR inhibitors, a 326 bp fragment of the  $\beta$ -globin gene was co-amplified in parallel for all specimens ([Tjhie et al., 1994](#)). All precautions to prevent cross-contamination were taken. Amplification products were visualized by electrophoresis on a 1.5 % agarose gel containing ethidium bromide.

Serum samples were tested for IgM and IgG antibodies to *M. pneumoniae* by an indirect IFA using a commercial kit (MP IFA; Zeus Scientific) as described previously ([Martínez et al., 2005](#)). Sera were adsorbed with an anti-human IgG reagent (Zorba; Zeus Scientific) prior to IgM testing.

**Interpretation.** A definitive diagnosis of *M. pneumoniae* infection was based on: (i) a demonstration of seroconversion, defined as a change from a negative acute serum sample to a positive convalescent serum sample or a fourfold rise in antibody titres between the paired sera; and (ii) a positive PCR result. A presumptive diagnosis of acute *M. pneumoniae* infection was made in patients from whom only the acute-phase serum was available and was based on the presence of an IgM titre  $\geq 32$ .

**Statistical analysis.** Sensitivity, specificity and predictive values were determined by standard procedures. Differences in the frequency of *M. pneumoniae* infections according to the patient's age and to the year of study were compared by Student's *t*-test and  $\chi^2$  tests, respectively. Statistical significance was defined by a two-sided  $\alpha$ -level of 0.05.



## RESULTS AND DISCUSSION

During our 36-month prospective study, 357 patients with pneumonia met the inclusion criteria for enrolment. There were 192 males (53.8 %) and 165 females (46.2 %); the patients' ages ranged from 18 to 94 years (median 63 years). In 232/357 (65.0 %) cases, paired sera were available.

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Overall, *M. pneumoniae* was diagnosed in 32/357 (9.0 %) patients. Patients positive for *M. pneumoniae* had a median age of 45 years (range 18–87 years), which was not significantly different to the median age (65 years) of *M. pneumoniae*-negative patients ( $P=0.1$ ). Among the 32 patients positive for *M. pneumoniae*, 23 (71.9 %) were found to be positive by PCR and 27 (84.4 %) by serology. Table 1\* summarizes the results of the comparison of PCR and serological results.

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**Table 1.** *M. pneumoniae* detection by PCR and serology in adults with CAP in Santiago, Chile, from February 2005 to January 2008

Acute and convalescent serum specimens were available in 21 out of 27 patients with positive serological results and therefore these cases could be confirmed as current *M. pneumoniae* infections. Of these 21 cases, three patients experienced only IgM seroconversion, 12 patients showed IgG seroconversion and six cases had both IgM and IgG seroconversion. Diagnosis of *M. pneumoniae* infection is usually confirmed by serological methods, because DNA amplification techniques are still not widely available in clinical laboratories. Additionally, the detection of a significant rise in specific immunoglobulin always confirms the diagnosis of a current infection. However, as shown in this study, serology is not useful for the early diagnosis of *M. pneumoniae* infection in adults. Adults may fail to develop IgM in the course of a *M. pneumoniae* infection, probably as a result of re-infection ([Sillis, 1990](#); [Uldum et al., 1992](#); [Jacobs, 1993](#)), or the IgM response may be delayed, not being detectable until 15 days after the onset of symptoms ([Jacobs et al., 1986](#); [Moule et al., 1987](#)). Thus the absence of IgM in the first serum sample does not exclude a current infection. In the present study, only seven of the 21 positive patients (33.3 %) for whom both serum samples were available were IgM positive in the acute-phase sera.

The serological diagnosis of the remaining six patients was supported by a single IgM titre  $\geq 32$  and represented presumptive *M. pneumoniae* infection; one of these patients also had a positive PCR result, which confirmed the result, but in the other five patients the IgM finding was the only laboratory evidence of infection. These cases highlight the limitations of establishing a serological diagnosis on a single serum sample. An elevated IgM titre is a reliable indicator of current *M. pneumoniae* infection in children ([Petitjean](#)

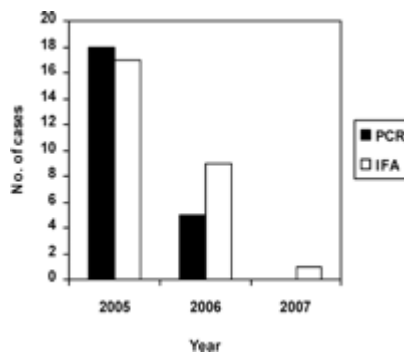
[et al., 2002](#)), but persistence of IgM following infections has been observed in adults, which is another limitation of the IgM-based diagnosis ([Sillis, 1990](#)). There is no reference technique for the serodiagnosis of *M. pneumoniae*. The IFA currently available commercially is a classical *M. pneumoniae* diagnostic procedure. It is more sensitive than the old complement fixation test ([Kok et al., 1989](#)) and shows 97.1 % agreement with the ImmunoCard (Meridian) assay and 91.4 % agreement with  $\mu$ -capture ELISA ([Barker et al., 1990](#); [Alexander et al., 1996](#)). In Chile, most hospitals perform IFAs for respiratory virus diagnosis as well as for diagnosis of *M. pneumoniae*; the test takes only 2 h to get results and is three to four times less expensive than other tests available commercially.

Good agreement was found between the results of PCR and serology, with corresponding negative and positive results in 325 (91.0 %) and 18 patients (5.0 %), respectively. Among the discordant results, five patients were PCR positive but serologically negative. The mean age of these patients was 72.4 years. In two of these cases, patients failed to develop an antibody response in the convalescent-phase serum, which could be explained by a deterioration of the immune response due to ageing ([Dorigo-Zetsma et al., 2001](#)). Alternatively, these cases may represent asymptomatic carriage of *M. pneumoniae* due to persistence from a previous disease ([Daxboeck et al., 2003](#)). In the other three patients, only the first serum sample was available so a late antibody response could not be ruled out ([Daxboeck et al., 2003](#)). Four patients showed IgG seroconversion, but had negative results in the PCR. These four cases probably represent false-negative PCR results. As the presence of inhibitors was ruled out in the PCRs, a low bacterial load resulting from previous antimicrobial treatment or dilution of the sample in the throat wash below the limit of detection could explain the negative PCR results. The type of respiratory specimen could affect the diagnostic efficacy of *M. pneumoniae* ([Räty et al., 2005](#)), and sputum seems to be better than other specimens, including throat washes, for the diagnosis of *M. pneumoniae* in CAP cases ([Kleemola et al., 1990](#); [Dorigo-Zetsma et al., 2001](#); [Räty et al., 2005](#)). By using serology as the gold standard, the sensitivity, specificity, and positive and negative predictive values of the PCR were 66.7, 98.5, 78.3 and 97.3 %, respectively.

Several studies have demonstrated that PCR is a good alternative to serological tests for the detection of *M. pneumoniae* in respiratory specimens from patients with CAP ([Tjhie et al., 1994](#); [Falguera et al., 1996](#); [Dorigo-Zetsma et al., 2001](#)). Several gene targets have been used for DNA amplification of this organism, with the P1 cytoadhesin and the 16S rRNA genes being the most commonly used ([Tjhie et al., 1994](#); [Falguera et al., 1996](#); [Dorigo-Zetsma et al., 2001](#)). In the present study, the global agreement between the PCR and IFA results was 343/357 (96.1 %). [Tjhie et al. \(1994\)](#), using the same primers and PCR conditions in adults with respiratory tract infections, found 88.1 % correlation between PCR and serology, the latter tested using a microparticle agglutination assay (Serodia Myco II). These differences may be explained by differences in the number of patients enrolled in the study, the proportion of paired sera analysed and differences in the serological tests employed, as well as in the type of illnesses.

*M. pneumoniae* infections are endemic worldwide. However, a cyclic pattern of epidemics, at intervals of 4–8 years, has been described in Asia, Europe and the USA ([Foy, 1993](#); [Sasaki et al., 1996](#); [Lind et al., 1997](#); [Rastawicki et al., 1998](#); [Layani-Milon et al., 1999](#)). In the present study, the overall incidence of *M. pneumoniae* in CAP cases

was 9.0%. The frequency of infections varied over the 3 years studied, which suggested the presence of an epidemic period during the study. During 2005, 16.3 % (21/129) of *M. pneumoniae* cases were detected, followed by 8.7 % (10/115) during 2006 and 0.9 % (1/113) in 2007 ( $P < 0.005$ ). Thus it will be necessary to undertake a longer period of surveillance to determine the periodicity of epidemics, as well as to determine the endemic level of *M. pneumoniae* infections in Chile. Fig. 1 compares the number of CAP cases associated with *M. pneumoniae* determined by PCR and serology over the 3 years of this study. During the first year, PCR and serology detected similar numbers of cases and the agreement between positive cases was 14/21 (66.7 %). However, during the second year, PCR detected a lower number of cases and the agreement between positive cases dropped to 40.0 % (4/10). The apparent difference in the diagnostic values during periods of low and high incidence of *M. pneumoniae* infection is worth further study.



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**Fig. 1.** *M. pneumoniae* detection by PCR and serology (IFA) in adults with CAP. Annual distribution in Santiago, Chile, from February 2005 to January 2008.

In conclusion, although a good global agreement was found between PCR and serology, the lower sensitivity of the PCR found in this study leads us to recommend the use of both procedures in parallel to confirm *M. pneumoniae* in CAP in adults.

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