Seroprevalence of *Mycoplasma pneumoniae* in HIV-infected patients using a microparticle agglutination test

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Mycoplasma pneumoniae is increasingly recognized as a common and important pathogen in community settings, and is responsible for various pulmonary and extrapulmonary conditions in the normal population. However, the seroepidemiology of acute *M. pneumoniae* infection in HIV-infected individuals is still unclear worldwide. This study examined the seroprevalence of antibodies to *M. pneumoniae* in HIV-infected patients admitted with respiratory complaints at a tertiary AIDS care centre in Chennai, India. A commercial gelatin microparticle agglutination test (Serodia-Myco II, Fujirebio) was used for the determination of antibodies against *M. pneumoniae* in acute serum specimens. Of the 200 HIV-infected patients with underlying pulmonary conditions tested, 34 (17 % positivity; 95 % CI 12–23 %) had antibodies specific to *M. pneumoniae*, while among the 40 patients with no underlying pulmonary symptoms, five (12.5 % positivity; 95 % CI 4–27 %) had evidence of anti-*M. pneumoniae* antibody. This shows that the incidence of *M. pneumoniae* seropositivity is greater in patients with underlying pulmonary complaints. Most positive titres were found in the age group 28–37 years in the symptomatic and symptom-free groups (64.7 and 60 %, respectively). The positive titres ranged from 40 to >20 480. High titres (≥ 320) were found in 10 out of the 39 patients (25.6 %). This seroprevalence study reports a 16.2 % prevalence of *M. pneumoniae* infections in HIV-infected patients by a particle agglutination test.

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INTRODUCTION

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 (Cassell *et al.*, 1981; Maniloff *et al.*, 1992). Laboratory diagnosis of *M. pneumoniae* infection has relied mainly on serologic tests, because the organism is difficult to isolate (Chamberlein *et al.*, 1983; Cimolai *et al.*, 1996; Fedorko *et al.*, 1995; Karppelein *et al.*, 1993; Tully *et al.*, 1979). A reliable and sensitive serologic test is needed for use in the early phase of infection by *M. pneumoniae* to confirm the infection and to ensure that the appropriate antibiotic is used for treatment. The detection of specific IgM, which appears 7–10 days after infection and approximately 2 weeks before IgG, has been shown to indicate a recent or current *M. pneumoniae* infection (Jacobs, 1993; Matas

et al., 1998; Sherman *et al.*, 1993; Sillis, 1990). 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 minimal or undetectable in some cases of adult reinfection with *M. pneumoniae* (Chamberlein *et al.*, 1983; Jacobs, 1993; Sherman *et al.*, 1993; Vikerfors *et al.*, 1988). 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 (Vikerfors *et al.*, 1988), approximately 20 % of adults did not mount an IgM response after infection with *M. pneumoniae*.

The seroepidemiology of acute *M. pneumoniae* infection in HIV-infected individuals is still unclear worldwide. Therefore, we tested serum specimens from HIV-infected adult patients with underlying respiratory complaints using the Serodia-Myco II gelatin particle agglutination test for detecting antibodies against *M. pneumoniae*. This test is

Abbreviation: CI, confidence interval.

based on the principle that sensitized particles are agglutinated by the presence of antibodies to *M. pneumoniae* in human serum.

METHODS

Patients. Sera from 200 HIV-infected patients admitted to the inpatient department of YRG-CARE, Chennai, Southern India, with underlying respiratory complaints and 40 HIV-infected patients without any pulmonary symptoms during a 1-year period (December 2004 to November 2005) were examined for *M. pneumoniae* antibody. The study was carried out by protocols approved by the Institutional Review Board (IRB) of YRG CARE, University of Madras, for ethical issues. Written, informed consent was obtained from all patients or their legal representatives before study enrolment. The study was carried out in compliance with good clinical practice, including the International Conference on Harmonization Guidelines and the Declaration of Helsinki.

This was a prospective cohort study, in which the patients were initially screened for HIV infection using HIV double ELISA (HIV-1 and -2), and later confirmed using Western blot assay (positive for HIV-1 antibody) (Immunetics) against gp160 env, gp120 env, p66 pol, p55 gag, gp41 env, p24 gag and p17 gag. Patients that underwent a clinical and radiological diagnosis of respiratory disease and who had new infiltrates on chest X-ray or consolidation which could not be attributed to some other aetiology, in addition to the following symptoms: cough, malaise, fever, sore throat with signs of sputum, dullness, rales/wheezes or evidence of pulmonary consolidation and myalgia, were considered. The body temperature, pulse rate, blood pressure and SPO₂ were recorded from all the patients upon admission. Patients with evidence of nosocomial pneumonia, lung cancer, aspiration pneumonia or bronchiectasis, and those terminally ill were excluded from the study.

Serologic testing. For serologic testing, a single acute blood specimen (3–5 ml) was collected aseptically from all the patients. The sera were separated and stored at –20 °C until tested. Prior to testing, samples were thawed and then centrifuged at 3000 g for 10 min.

Serodia-Myco II is an *in vitro* diagnostic test for the detection of antibodies to *M. pneumoniae* which is manufactured using artificial gelatin particles sensitized with cell-membrane components of *M. pneumoniae* (Mac strain). It is based on the principle that the sensitized gelatin particles are agglutinated in the presence of antibodies against *M. pneumoniae*. Briefly, 25 µl of serum was diluted twofold to give dilutions of 1 in 10 to 1 in 10 240. Sensitized and unsensitized particles were added to the 1 in 10 serum dilution to give a final dilution of 1 in 20. Drops (25 µl) of the sensitized particle suspension were then added to the remaining wells, giving final dilutions of 1 in 40 to 1 in 20 480. The plates were shaken for 30 s and then covered and left undisturbed on a level surface at room temperature for 3 h (Ikeda & Omori, 2004; Srifuengfung *et al.*, 2004).

The test was initially calibrated using the control serum dilution series. Each batch of tests included control wells containing 25 µl of diluent, 25 µl of the particle suspensions and dilutions of a reactive control serum of known titre, supplied with the commercial kit.

Buttons or compact, smooth rings of particles in the bottom of the wells were read as negative agglutination patterns, and a more extensive ring as positive. Titres ≥ 40 were regarded as positive for *M. pneumoniae* antibody.

Standard statistical procedures were adopted to reveal significant findings.

RESULTS

Serum specimens from 240 HIV-infected patients were screened for the presence of antibodies against *M. pneumoniae*; 200 patients had underlying respiratory conditions, while 40 patients formed the control group without any pulmonary symptoms. Table 1 shows the results of 240 serum specimens tested by Serodia-Myco II particle agglutination. Of the 200 HIV-infected patients with underlying pulmonary conditions tested, 34 (17 % positivity; 95 % CI 12–23 %) had antibodies specific to *M. pneumoniae*, while among the 40 patients with no underlying pulmonary symptoms, five (12.5 % positivity; 95 % CI 4–27 %) showed evidence of anti-*M. pneumoniae* antibody. This shows that the incidence of *M. pneumoniae* seropositivity was greater in patients with underlying pulmonary complaints. Table 2 shows that the positive titres ranged from 40 to $>20\,480$. High titres (>320) were found in 10 of the 39 patients (25.6 %). The highest titre of 20 480 was found in two (5.1 %) patients, one with respiratory disease and another with no underlying pulmonary ailments. There was no significant difference between the two groups with respect to seropositivity and sex ($z=0.06$, $P=0.95$). ($P>0.05$ was considered significant). However, there was a significant difference with regard to sex and seropositivity among those without respiratory complaints ($z=3.1$, $P=0.002$). In the HIV-infected subjects with no respiratory complaints, the incidence of seropositivity was 16 % ($n=4$) in females and 7 % ($n=1$) in male subjects. The overall positivity of antibodies specific to *M. pneumoniae* in the HIV-infected patients tested was 16.2 %.

DISCUSSION

AIDS is manifested as a collection of diseases resulting from severe immunosuppression due to HIV infection. AIDS is clinically defined as the stage when an HIV-infected person's CD4+ lymphocyte count falls below 200 per microlitre or the HIV-infected person has any of the AIDS-defining clinical illnesses. Mycoplasma infection is highly significant clinically in AIDS, even if it is only one example of an opportunistic infection, as opportunistic infections are reportedly the direct cause of death in more than 80 % of patients with AIDS (Blanchard & Montagnier, 1994).

Although studies to determine the prevalence of *M. pneumoniae* in various parts of the world have been carried out, there is still a paucity of good epidemiological data on the frequency with which atypical pathogens such as *M. pneumoniae* cause community-acquired pneumonia in India (Shankar *et al.*, 2005). Isolation of *M. pneumoniae* from atypical bacterial pneumonia is considered to be the gold standard for diagnosis. However, isolation requires 2–3 weeks, which limits its clinical usefulness. Serology is probably the most frequently used method to diagnose *M. pneumoniae* infections. Moreover, serologic tests are more sensitive indicators of mycoplasma infection than culture of the organism, because in many instances antibody responses can be detected when the organism cannot

Table 1. *M. pneumoniae*-positive serum from HIV-infected patients with and without respiratory complaints of different age groups

The number of patients with a positive serum result is shown.

Patient details	Age (years)					Total
	18–27	28–37	38–47	48–57	> 57	
With respiratory complaints						
Male (<i>n</i> = 161)	1	16	6	2	3	28
Female (<i>n</i> = 39)	—	6	—	—	—	6
Total (<i>n</i> = 200)	1	22	6	2	3	34
Percentage positive	2.9	64.7	17.6	5.8	8.8	100.0
Without respiratory complaints						
Male (<i>n</i> = 25)	—	2	2	—	—	4
Female (<i>n</i> = 15)	—	1	—	—	—	1
Total (<i>n</i> = 40)	—	3	2	—	—	5
Percentage positive	—	60.0	40.0	—	—	100.0

be recovered (Srifuengfung *et al.*, 2004). Therefore, current routine methods for laboratory diagnosis of *M. pneumoniae* infection are based primarily on serologic analysis (Srifuengfung *et al.*, 2004; Hammerschlag, 2001). Among the various serological tests available for detecting *M. pneumoniae* infections, ELISA has been described to be the most sensitive and specific (Marmion *et al.*, 1993; Clyde, 1983; Kleemola *et al.*, 1982). We tested a single serum sample from each patient, as this has been reported to be of epidemiologic value and helps in determining the percentage of a population that has actually been exposed to the organism (Velleca *et al.*, 1980).

In addition, the complement fixation test (CFT), a commonly used test, uses a lipid component of *M. pneumoniae* that cross-reacts with components of mammalian tissue as well as bacterial glycolipids, giving false-positive results (Kenny *et al.*, 1990; Jacobs *et al.*, 1986; Brunner *et al.*, 1977;

Dussaix *et al.*, 1983). The CFT is also limited by its inability to detect specific antibodies other than IgG (Gendrel *et al.*, 1997). Numerous authors have described serology as the standard laboratory method for the diagnosis of *M. pneumoniae* infections (Sillis, 1990). Therefore, our study used serology to look for the seroprevalence of *M. pneumoniae* infections among HIV-infected subjects. This study was aimed primarily at determining the seroprevalence rate of *M. pneumoniae*, rather than diagnosing the aetiological agent in HIV-infected individuals. However, information on the reported cross-reactivity between *M. pneumoniae* and *Mycoplasma genitalium* was not available for this test, although this is recognized to be a problem, particularly with the CFT.

Gendrel *et al.* (1997) diagnosed *M. pneumoniae* infection in 42% of patients. An Indian study has reported that the prevalence rate of *M. pneumoniae* in the normal population

Table 2. Positive serum from HIV-infected patients of different age groups

Titre	Number of positive serum samples (<i>n</i> = 39)			Percentage of positive serum samples
	Male (<i>n</i> = 186)	Female (<i>n</i> = 54)	Total (<i>n</i> = 240)	
40	8	1	9	23.0
80	9	5	14	35.8
160	5	1	6	15.3
320	4	—	4	10.2
640	—	—	—	—
1280	1	—	1	2.5
2560	1	—	1	2.5
5120	1	—	1	2.5
10 240	1	—	1	2.5
20 480	2	—	2	5.1
> 20 480	—	—	—	—
Total	32	7	39	100.0

is about 35 % (Dey *et al.*, 2000), while others have identified it in only 1·9 % of patients (Mundy *et al.*, 1998). Nevertheless, the role of *M. pneumoniae* in adults with respect to HIV infection needs to be defined.

According to our criteria, 17 % (95 % CI 12–23 %) of those with underlying pulmonary conditions had antibodies specific to *M. pneumoniae*, while five (12·5 %; 95 % CI 4–27 %) of the 40 patients with no underlying pulmonary symptoms had evidence of anti-*M. pneumoniae*. Clyde (1983) has shown that *M. pneumoniae* accounts for up to 30 % of all pneumonia cases in the normal population. No significant difference could be established between the two groups with respect to seropositivity and sex ($z=0\cdot06$, $P=0\cdot95$) in the present study. Nevertheless, a significant difference was demonstrated with regard to sex and seropositivity among those without respiratory complaints ($z=3\cdot1$, $P=0\cdot002$); this aspect needs further study. The prevalence of *M. pneumoniae* in HIV-infected individuals seems to contradict reports that *M. pneumoniae* is commonly seen in children and young people rather than adults, as our study was carried out in adult patients with HIV. Evidence of infection was a titre of 1:40 or greater. However, the specification for this assay did not state whether it detected IgG or IgM. Moreover, the present finding should not be considered to reflect the exact seroprevalence of antibodies against *M. pneumoniae*, as recent studies have shown that HIV-infected adults may not produce measurable IgG against *M. pneumoniae* (Twigg *et al.*, 1996). In addition, patients continue to produce detectable levels of IgM occasionally, due to incomplete clearance of the organism from the respiratory tract (Atkinson *et al.*, 2004; Twigg *et al.*, 1996).

In conclusion, antibody specific to *M. pneumoniae* was detected in 16·2 % of the HIV-infected patients screened. To the best of our knowledge, this study is the first to report the seroprevalence of *M. pneumoniae* among HIV-infected patients.

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