ORIGINAL ARTICLE

Prevalence of *Mycoplasma Pneumoniae* Antibodies Among Malaysians

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Summary

Sera from 333 patients with pneumonia and 304 age-matched healthy controls were examined for antibodies to *Mycoplasma pneumoniae* by a particle agglutination test (SERODIA-MYCO II, Fujirebio Inc. Tokyo, Japan). Overall, 11.8% of controls and 25.5% of patients had titres ≥ 40 while 2% of controls and 18.6% of patients had titres of ≥ 80. The prevalence of antibodies among controls was highest among adolescents and appeared to diminish with age thereafter. Of the patients with titres ≥ 80, 61.3% were under 10 years old.

The results suggested that with the SERODIA-MYCO II test, 1:80 might be a suitable cut-off titre for the diagnosis of mycoplasmal pneumonia.

Key Words: Mycoplasma pneumoniae, antibody titres

Introduction

Mycoplasma pneumoniae causes a variety of respiratory as well as extra-pulmonary infections including pharyngitis, tracheobronchitis, pneumonia, meningitis and arthritis[1]. These infections can be diagnosed by isolating the organism or demonstrating the presence of specific antigens or DNA. However, isolation of the organism requires 7 to 10 days' incubation which is too slow for therapeutic considerations. More rapid antigen or DNA detection assays are still not readily available for use in routine diagnostic laboratories. Most often, diagnosis is made by serology, based on the demonstration of significant antibody titres.

The most frequently used serological test is the complement fixation test (CFT) which uses a glycolipid extract that shows cross-reacting epitopes with other bacteria and with host cellular antigens[2]. Hence false positive titres are common. Moreover, CF antibodies may be absent in re-infections thus causing false negatives in older patients.

Serodiagnosis should be based on a significant increase or decrease in antibody titres. Unfortunately, paired sera are not available from most Malaysian patients and diagnosis often has to be made on a single antibody titre. The interpretation of serology is complicated by the fact that following an infection, antibodies, particularly IgG, may persist for some time making it difficult to differentiate past from recent infection. Fortunately, patients with recent infections tend to have higher antibody titres than people with past exposure. Hence it is possible to establish a cut-off titre which would help to distinguish recent infection from past exposure.

The aim of this study is to determine the prevalence of *M pneumoniae* antibodies among healthy Malaysians and to compare the antibody titres among infected individuals and healthy controls so as to establish a suitable cut-off titre for diagnosing *M pneumoniae* infections in Malaysia.

Materials and Methods

Study subjects

Sera were collected from 304 healthy asymptomatic children and adults who took part in a community hepatitis B survey carried out in Selangor in 1993 and 333 patients with clinical and chest X-ray signs of pneumonia, seen at the University Hospital, Kuala Lumpur, from November 1993 to April 1994. The sera were stored for varying periods up to 9 months, at -20°C, before testing.

Antibody detection

Antibodies to *M pneumoniae* were detected and quantitated using a SERODIA-MYCO II test kit (Fujirebio Inc., Tokyo, Japan), following the manufacturer's instructions. This is a particle agglutination test using artificial gelatin particles sensitised with cell membrane components of *M pneumoniae* as antigen. Sera were diluted from 1:40 to 1:5120 and the results were read after 3 hours' incubation at room temperature. Positive and negative controls were included in each run.

Statistical significance was evaluated by the x^2 test.

Results

The *M pneumoniae* antibody titres of healthy asymptomatic subjects and patients with clinical signs of pneumonia obtained in this study are shown in Tables I and II.

Of the healthy individuals, only 36 (11.8%) were positive for antibodies at ≥ 40. The highest titre obtained was only 160. The prevalence of antibodies was highest among adolescents and appeared to diminish with age thereafter.

Among patients, 85 (25.5%) had titres ≥ 40 which is significantly ($p<0.01$) more than in the healthy controls (11.8%). The highest titres were >2560. In every age group, except the 30-39 year-olds, the percentage of individuals with a titre of ≥ 40 is significantly higher for patients than for the control population ($p<0.01$ - <0.5). At a titre of ≥ 80, 18.6% of patients were seropositive compared to 2% of controls. Only 23 paired serum samples (6.9%) were collected from the 333 patients. Of these, 18 (78.3%) were considered seropositive because of high titres or significant changes in titre.

Discussion

There is an inevitable overlap in antibody titres between people with past exposure to an infection and those suffering the acute infection. For the diagnosis of infection from a single serum sample, the cut-off titre is usually taken to be the titre exceeded by no more than 15% of the control population[3]. As the level of antibodies in control populations differs in

Table I
Age-related antibody prevalence in healthy Malaysians

Age (years)	Antibody titres				No. tested	No.(%) with ≥ 40
	< 40	40	80	160		
0 - 9	45	4		1	50	5 (10)
10 - 19	44	9	1	1	55	11 (20)
20 - 29	42	7		1	50	8 (16)
30 - 39	43	6			49	6 (12.2)
40 - 49	48	1	1		50	2 (4)
≥ 50	46	3	1		50	4 (8)
Total	268	30	3	3	304	36 (11.8)

Table II
Age-related antibody prevalence among patients with pneumonia at the University Hospital, K.L.

Age (years)	Antibody titres <40	40	80	160	320	640	1280	≥ 2560	No. tested	No.(%) ≥ 40	No.(%) ≥ 80
0 – 9	106	13	11	4	7	7	6	3	157	51 (32.5)	38 (24.2)
10 – 19	19	3	1	1	2	1			27	8 (29.6)	5 (18.5)
20 – 29	21	2	2		1	1	1	2	30	9 (30.0)	7 (23.3)
30 – 39	21	1			1				23	2 (8.7)	1 (4.3)
40 – 49	13	1			1			1	16	3 (18.8)	2 (12.5)
≥ 50	27		3		1		1		32	5 (15.6)	5 (15.6)
Unknown	41	3	2	2					48	7 (14.6)	4 (8.3)
Total	238	23	19	7	13	9	8	6	333	85 (25.5)	62 (18.6)

different communities, it is essential that the recommended cut-off titre is established for the appropriate control population.

The manufacturer of SERODIA-MYCO II recommends ≥ 1:40 as a positive titre for mycoplasmal infection. In this study, 4-20% of healthy controls in different age groups had this titre. Applying 1:40 as the cut-off titre for all age groups would cause overdiagnosis particularly among those 10-39 years old. On the other hand, infected patients do not always show high antibody titres. From the 23 paired sera we received, 28% had a titre of ≤ 40 in the acute phase sample. While 4 to 64-fold increases in titre were usual, about a quarter of the increases were from <40 to 80 only. Hence a substantial number of infections in patients may be missed if paired sera were not available and >1:80 were taken to be the cut-off titre. The difficulty with establishing a cut-off titre from our seroprevalence data was further compounded by the lack of confirmation tests for mycoplasmal infection in our patients. We also could not exclude other bacterial and viral causes of pneumonia from all our patients. As such, we could not be sure that all patients with arbitrarily defined "positive" titres truly had mycoplasmal infection.

Using the SERODIA-MYCO II test, other Malaysian workers[4,5] have found 15-45% seropositives (≥ 1:40) among healthy controls and 27.9-40.5% positives (at titres of ≥ I:160 and ≥ I:40 respectively) among patients with respiratory infections. Our results indicated that I:80 was the most suitable cut-off titre for the diagnosis of mycoplasmal pneumonia in Malaysians. At this titre, overall, 18.6% of our pneumonia patients possibly had a mycoplasmal infection (from 4.3% among 30-39 year-olds to 24.2% among paediatric patients). As in other countries[6], the incidence of mycoplasmal infections in Malaysia also appears to be highest among children and young adults.

For the diagnosis of mycoplasmal infection in individual patients more comprehensive testing should be employed wherever possible. The SERODIA-MYCO II test detects both IgG and IgM. The presence of IgM usually indicates recent infection and separate testing for mycoplasmal IgM is possible with indirect immunofluorescence and IgM capture enzymeimmunoassays. However, reinfections with *M pneumoniae* are reported to be common[6] and in reinfections, IgM is often not detectable. Like IgM, IgA also declines to insignificant levels within a few months after infection. Therefore when paired sera are not available, concurrent testing for IgG, IgM and IgA can help to distinguish recent from past infection.

Acknowledgement

This study is supported by IRPA grant no. 3-07-04-102.

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