Neurological disease associated with *Mycoplasma pneumoniae* infection. PCR evidence against a direct invasive mechanism

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Abstract

Aims—To investigate the pathology in patients presenting with sudden onset neurological illnesses associated with *Mycoplasma pneumoniae* infection.

Methods—*M pneumoniae* infection was diagnosed by a highly rigorous interpretation of serological markers initially using complement fixation, agglutination and IgM antibodies. Confirmation of the serological diagnosis was achieved using indirect immunofluorescence for IgM, IgA, and IgG. Serum and cerebrospinal fluid (CSF) samples from these patients were examined using the polymerase chain reaction to look for evidence of *M pneumoniae* DNA.

Results—No *M pneumoniae* DNA was found in any serum or CSF samples. Diagnosis of *M pneumoniae* infection by agglutination and complement fixation antibodies was not always confirmed by indirect immunofluorescence.

Conclusion—The neurological lesions in these patients do not appear to be caused by the direct invasion of *M pneumoniae* into the nervous system. The lesions may be an immune response to infection. Serological diagnosis of *M pneumoniae* continues to be a laboratory problem.

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Mycoplasma pneumoniae infection in the United Kingdom occurs in epidemics lasting about 18 months, in four year cycles and with sporadic cases occurring in the community between epidemics. It is primarily a pathogen of the respiratory tract causing illnesses ranging from mild upper respiratory tract symptoms to severe pneumonia and occasional unexpected death in young fit individuals.¹⁻³ *M pneumoniae* disease is not commonly recorded in those aged under five years, where it may be clinically inapparent,⁴ or in the elderly.⁵ It has been associated with a spectrum of non-respiratory disease in children and adults including central and peripheral nervous system lesions,⁶ haemolytic anaemia⁷ and various skin manifestations, such as maculopapular rash and erythema-multiforme,⁸ generally appearing 12 to 21 days after a respiratory infection. These extrapulmonary signs are believed to be immune mediated phenomena although there are rare reports of culture of the organism from skin^{8,9} and cerebrospinal fluid (CSF).¹⁰

The pathological processes in non-respiratory *M pneumoniae* disease remain hypothetical. An induction of autoantibodies,¹¹ changes in the glycoproteins on the red cell membrane^{12,13} and perivascular infiltration around blood vessels¹⁴ are all suggested. The pathogenic process in respiratory disease is also still a matter for speculation.¹⁵

A major problem in the diagnosis of *M pneumoniae* infection is that conflicting laboratory results may occur.^{16,17} The polymerase chain reaction (PCR) can be a very sensitive technique for detecting any mycoplasma DNA in clinical samples¹⁸ and offers the possibility of quicker diagnosis earlier in the course of the disease and is a potential refinement to any investigation of the pathological processes.

Methods

SELECTION CRITERIA

Patients were selected from those presenting with acute and unexplained neurological symptoms in the Oxford region, at the Coventry and Warwickshire Hospital, Coventry, and the General Hospital, High Wycombe (tables 1 and 2). Of the 11 patients, three had sixth nerve palsy, two had third nerve palsy, two had transverse myelitis, one had optic neuritis, one had choreiform movements, one had Guillain-Barre syndrome, and one had a right-sided hemiplegia. They were screened on referral for enteroviruses, Epstein-Barr virus, mumps, measles, cytomegalovirus, herpes simplex virus types 1 and 2, and varicella zoster virus using serology and isolation techniques as appropriate for the organisms screened. Patients with evidence of viral or recent bacterial infections, including Q fever, Legionella and psittacosis, were excluded from this study.

LABORATORY DIAGNOSIS OF MYCOPLASMA PNEUMONIAE INFECTION

An initial suspicion of recent infection with *M pneumoniae* was based on clinical presentation, together with an agglutinating antibody titre of 100 or greater to mycoplasma antigen, using a Serodia-Myco-II gelatin particle agglutination test (Fujirebio, Japan). A further agglutination titre at this level or an increase to this titre or above in a second serum sample at least seven days later was considered significant. A complement fixation antibody (Behringwerke AG, Marburg, Germany) was used in conjunction with the agglutination test as a further marker of possible recent infection, taking a minimum antibody level of 1/128 in the first serum sample or this level in both samples, or a fourfold

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Table 1 Patients with neurological lesions and serological and sero-immunofluorescent evidence of *M pneumoniae* infection

Patient initials	Age (years)	Sample type	CFT result	Agglutination	Antibody titre			ELISA (IgM)	Clinical presentation
					IgM	IgG	IgA		
LC	5	Serum	512	320	>16	>64	>16	Positive	URTI and third nerve palsy
		Serum	512	320				Positive	
HC	6	Serum	256	320	8	64	<4	Positive	Febrile illness and episodes of right-sided hemiplegia
		Serum	256	320				Positive	
		CSF	<2	—				Positive	
CO	2	Serum	256	640	<4	32	4	Positive	Febrile illness then sixth nerve palsy
		CSF	<2	—				Negative	
RR	17	Serum	>512	>1280	>16	>128	>16	Positive	Pneumonia followed by transverse myelitis
		CSF	—	—				Positive	
FW	5	Serum	256	340	>16	>128	>16	Positive	Cerebellar ataxia
		Serum	256	340				Positive	
DB	9	Serum	128	40	<4	16	<4	Positive	Febrile illness followed by Guillain-Barre syndrome
		Serum	256	160				Positive	
		Serum	256	160				Positive	
RB	9	Serum	32	320	>16	8	32	Positive	Febrile illness and third nerve palsy
		Serum	16	320				Positive	

CFT = complement fixation test.

Table 2 Patients with neurological lesions but evidence of *M pneumoniae* infection

Patient initials	Age (years)	Sample type	CFT result	Agglutination	Antibody titre			ELISA (IgM)	Clinical presentation
					IgM	IgG	IgA		
CW	4	Serum	16	100	<4	<4	<4	Positive	Febrile and sixth nerve palsy
		CSF	<8	<40				Positive	
EP	4	Serum	128	<40	<4	<4	<4	Positive	URTI and transverse myelitis
		Serum	128	<40				Positive	
HJ	9	Serum	128	40	<4	<4	<4	Positive	Bilateral optic neuritis
		Serum	128	40				Positive	
NS	7	Serum	256	320	<4	<4	<4	Positive	Sixth nerve palsy
		Serum	256	320				Positive	

CFT = complement fixation test.

increase in the antibody titre between the first and second serum samples.

A μ -capture enzyme linked immunosorbent assay for IgM specific to *M pneumoniae* (Dia- tech Diagnostica Ltd., Israel) was used as a further marker of infection demonstrating IgM in one or both serum samples in some patients. Confirmation of the presumptive diagnoses of *M pneumoniae* was undertaken using an indirect immunofluorescence assay system to detect IgG, IgA and IgM.^{16 17}

LABORATORY CRITERIA FOR PATIENT INCLUSION
Patients were included in this study if they fulfilled a minimum of one of the following criteria: complement fixation antibody for *M pneumoniae* ≥ 128 and *M pneumoniae* IgM detected; complement fixation antibody for *M pneumoniae* with a fourfold increase in antibody titre between acute and convalescent serum samples; complement fixation antibody for *M pneumoniae* ≥ 128 and an agglutination titre ≥ 100 ; *M pneumoniae* agglutination titre ≥ 100 and detection of *M pneumoniae* specific IgM (All *M pneumoniae* IgM initially measured by μ -capture).

PCR FOR M PNEUMONIAE
A semi-nested PCR method using primers derived from the adhesin gene of *M pneumoniae* has been described previously.¹⁸ The sensitivity of this system is reported to be one DNA copy per sample and it has been shown to be greater than five logs more sensitive than antigen detection or culture.

PATIENT SPECIMENS EXAMINED
Cerebrospinal fluid was available from patients

where clinical investigation justified this invasive procedure. In some patients a second serum sample was not available and single or paired samples were tested. All clinical material underwent PCR amplification using primers specific for *M pneumoniae* and was tested without prior DNA extraction.¹⁸

Results
The clinical and serological details of patients who were initially considered to have been recently infected with *M pneumoniae* are presented in tables 1 and 2.

Of 11 patients initially selected on clinical and specific serological criteria, seven were confirmed, by indirect immunofluorescence, as being unequivocally recently infected with *M pneumoniae* (table 1). For the remaining four patients the diagnosis was not confirmed because the indirect immunofluorescence antibody assay did not support the complement fixation and/or agglutination test results (table 2).

M pneumoniae DNA was not detected by PCR in any of the serum or CSF samples examined.

Six of the seven patients with confirmed *M pneumoniae* infection and three of the remaining four patients reported a febrile illness or upper respiratory tract infection six to 14 days before the onset of neurological symptoms. All patients had made a symptomatic recovery within one year.

Discussion
Most authors believe that a clinical diagnosis followed by early and appropriate antibiotic

treatment¹⁹ hastens the clinical resolution of *M pneumoniae* associated respiratory disease.²⁰ There is still argument whether or not antibiotics influence the natural history of *M pneumoniae* non-respiratory disease. It has been suggested that the clinical manifestations of this infection may be mediated by the host immune system or a result of peroxide production by the organism.^{4,21}

In this study the resolution of the neurological lesions occurred over a variable time, but all had resolved within one year which is in agreement with earlier case reports.^{22,23} Other authors did not note the time of resolution of the lesions²⁴ and some recorded residual deficit years after infection.²⁵ The small number of patients in our study prevents any valid analysis of the effect of antibiotic treatment on the rate of clinical resolution of the neurological lesions.

In the cases recorded here, six of seven patients reported an episode of infection (upper respiratory tract infection (URTI) or fever) within 14 days of the onset of neurological signs. A prodrome of a respiratory infection 10 to 21 days before the onset of neurological disease is well described,¹⁶ but neurological lesions are known to appear without respiratory involvement²⁶ and without recorded fever.

An accurate laboratory diagnosis of *M pneumoniae* is important because of the variable clinical presentation of mycoplasma infection which often mimicks that of other common pathogens. Early and reliable diagnosis of infection is essential for any therapeutic advantage, to enable study of the disease process and for epidemiological information.

Laboratory diagnosis continues to be a problem: the organism is fastidious and slow growing and culture takes up to four weeks¹⁶ which is of limited value for clinical purposes. Sero-diagnostic methods also present problems because of the antigenic diversity of the organism which is larger than a virus, but has only about one fifth of the genetic material of a bacterium.²⁷ The complement fixation antibody titre may be late to rise after infection and may result from cross-reaction with other organisms or host material.²⁸ Agglutination tests can produce false positive results and there may or may not be an IgM response.¹⁶ An immunofluorescence assay to detect *M pneumoniae* specific IgM, IgA¹⁷ and IgG was chosen as the definitive test as it has been thoroughly evaluated in different patient populations and directly compared with respiratory culture results from the same patients. It is a labour intensive and skilled technique and is available only in specialist laboratories. Therefore, we used this test in this series as a final arbiter of infection, whilst applying a new and simple PCR *M pneumoniae* DNA detection system to gain more insight into mycoplasma associated pathology.

In contrast to a recently reported series²⁴ we applied strict serodiagnostic criteria in selecting patients for this study. Using these criteria for laboratory diagnosis of *M pneumoniae* infection we excluded four patients who may, in other series, have been assumed to have sustained neurological lesions associated with *M pneu-*

moniae infection. As there are many opinions on the criteria required for the laboratory diagnosis of *M pneumoniae*, PCR was performed on all serum and CSF samples from the 17 patients.

The PCR diagnostic system is an exquisitely sensitive and specific test for the detection of *M pneumoniae* specific DNA.¹⁸ Negative findings in this series adds weight to the theory that *M pneumoniae* associated neurological disease may be an immune mediated host response⁴ or possibly caused by release of toxic substances at the cell surface²¹ following elimination of the organism. Another possible mechanism for the neural pathology could be temporary occlusion of small blood vessels,²² a hypothesis advanced after observing the clinical development of third nerve palsy in one patient. This particular observation is supported by the reported pathology of perivascular inflammation observed in experimental *M pneumoniae* infection.¹⁴

A further *M pneumoniae* epidemic is expected in the United Kingdom in 1994–95. Collection of reliable epidemiological data relating to transmission of the organism will require sensitive and specific diagnostic methods with strict criteria for positivity. As PCR is a highly specific and very sensitive detection system, now simplified for routine use,¹⁸ data on the prevalence of *M pneumoniae* collected by population survey using PCR on throat swabs may test the observation that there may be passive carriage of the organism.²⁹ Such a survey together with defined serological diagnostic criteria may provide some insight into this enigmatic organism.

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