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.


Keywords: Mycoplasma pneumoniae, PCR, neurological lesions, pathogenesis.

*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 anaemia 7 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 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 and perivascular infiltration around blood vessels 13 are all suggested. The pathogenic process in respiratory disease is also still a matter for speculation. 14

A major problem in the diagnosis of *M pneumoniae* infection is that conflicting laboratory results may occur. 15-17 The polymerase chain reaction (PCR) can be a very sensitive technique for detecting any mycoplasma DNA in clinical samples 18 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
Table 1  Patients with neurological lesions and serological and sero-immunofluorescent evidence of M pneumoniae infection

<table>
<thead>
<tr>
<th>Patient initials</th>
<th>Age (years)</th>
<th>Sample type</th>
<th>CFT result</th>
<th>Agglutination</th>
<th>IgM</th>
<th>IgG</th>
<th>IgA</th>
<th>ELISA (IgM)</th>
<th>Clinical presentation</th>
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<td>LC</td>
<td>5</td>
<td>Serum</td>
<td>512</td>
<td>320</td>
<td>&gt;16</td>
<td>&gt;64</td>
<td>&gt;16</td>
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<td>URTI and third nerve palsy</td>
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<tr>
<td>HC</td>
<td>6</td>
<td>Serum</td>
<td>512</td>
<td>320</td>
<td>8</td>
<td>64</td>
<td>&lt;4</td>
<td>Positive</td>
<td>Febrile illness and episodes of right-sided hemiplegia</td>
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<tr>
<td></td>
<td></td>
<td>Serum</td>
<td>256</td>
<td>320</td>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td>Febrile illness and sixth nerve palsy</td>
</tr>
<tr>
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<td></td>
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<td>&lt;2</td>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>Pneumonia followed by transverse myelitis</td>
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<tr>
<td></td>
<td></td>
<td>CSF</td>
<td></td>
<td>640</td>
<td>&lt;4</td>
<td>32</td>
<td>4</td>
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<td>Cerebellar ataxia</td>
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<td>&gt;1280</td>
<td>&gt;16</td>
<td>&gt;128</td>
<td>&gt;16</td>
<td>Positive</td>
<td>Febrile illness followed by Guillain-Barre syndrome</td>
</tr>
<tr>
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<td></td>
<td>CSF</td>
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<td>&lt;2</td>
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<td></td>
<td>Positive</td>
<td>Febrile illness and third nerve palsy</td>
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<tr>
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<td></td>
<td>Serum</td>
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<td>&gt;1280</td>
<td>&gt;16</td>
<td>&gt;128</td>
<td>&gt;16</td>
<td>Positive</td>
<td>Febrile illness and sixth nerve palsy</td>
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<td>&gt;1280</td>
<td>&gt;16</td>
<td>&gt;128</td>
<td>&gt;16</td>
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<tr>
<td></td>
<td></td>
<td>CSF</td>
<td>&lt;2</td>
<td>&lt;2</td>
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<td></td>
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<td>Febrile illness and sixth nerve palsy</td>
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<td>&gt;128</td>
<td>&gt;16</td>
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<td>&lt;2</td>
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<td></td>
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<td>&gt;128</td>
<td>&gt;16</td>
<td>Positive</td>
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<td></td>
<td>Positive</td>
<td>Febrile illness and sixth nerve palsy</td>
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<td>&gt;1280</td>
<td>&gt;1280</td>
<td>&gt;16</td>
<td>&gt;128</td>
<td>&gt;16</td>
<td>Positive</td>
<td>Febrile illness and sixth nerve palsy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CSF</td>
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<td></td>
<td>Positive</td>
<td>Febrile illness and sixth nerve palsy</td>
</tr>
<tr>
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<td></td>
<td>Serum</td>
<td>&gt;1280</td>
<td>&gt;1280</td>
<td>&gt;16</td>
<td>&gt;128</td>
<td>&gt;16</td>
<td>Positive</td>
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<td>Positive</td>
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<tr>
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<td>Serum</td>
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<td>&gt;1280</td>
<td>&gt;16</td>
<td>&gt;128</td>
<td>&gt;16</td>
<td>Positive</td>
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<td>&lt;2</td>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td>Febrile illness and sixth nerve palsy</td>
</tr>
</tbody>
</table>

CFT = complement fixation test.

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

<table>
<thead>
<tr>
<th>Patient initials</th>
<th>Age (years)</th>
<th>Sample type</th>
<th>CFT result</th>
<th>Agglutination</th>
<th>IgM</th>
<th>IgG</th>
<th>IgA</th>
<th>ELISA (IgM)</th>
<th>Clinical presentation</th>
</tr>
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<tbody>
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<td>Serum</td>
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<td>100</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>Positive</td>
<td>Febrile and sixth nerve palsy</td>
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<td>EP</td>
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<td>Serum</td>
<td>&lt;8</td>
<td>&lt;40</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>Positive</td>
<td>URTI and transverse myelitis</td>
</tr>
<tr>
<td>HJ</td>
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<td>128</td>
<td>&lt;40</td>
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<td>Positive</td>
<td>Bilateral optic neuritis</td>
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<tr>
<td>NS</td>
<td>7</td>
<td>Serum</td>
<td>128</td>
<td>&lt;40</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>Positive</td>
<td>Sixth nerve palsy</td>
</tr>
</tbody>
</table>

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
Neurological disease associated M pneumoniae infection

is still biotics influence treatment but all had resolved within this infection 14 in respiratory tract 21 in vantage, system or a signs. of clinical resolution disease is which is clinical and epidemiological lesions occurred with earlier infection, 21 applying to a contrast to a material. 28 culture swabs can rise after infection in culture and is available for the time, record of residual manifestations of M pneumoniae disease. 20 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 response or possibly caused by release of toxic substances at the cell surface following elimination of the organism. Another possible mechanism for the neural pathology could be temporary occlusion of small blood vessels, 27 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. 79 Such a survey together with defined serological diagnostic criteria may provide some insight into this enigmatic organism.

17 Sillis M. The limitations of IgM assays in serological diag-