Neurological Symptoms in Patients Whose Cerebrospinal Fluid Is Culture- and/or Polymerase Chain Reaction–Positive for *Mycoplasma pneumoniae*

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We describe 13 patients with neurological signs and symptoms associated with *Mycoplasma pneumoniae* infection. *M. pneumoniae* was isolated from the cerebrospinal fluid (CSF) of 9 patients: 5 with meningoencephalitis, 2 with meningitis, and 1 with cerebrovascular infarction. One patient had headache and difficulties with concentration and thinking for 1 month after the acute infection. *M. pneumoniae* was detected, by means of PCR, in the CSF of 4 patients with negative culture results. Two had epileptic seizures, 1 had blurred vision as a consequence of edema of the optic disk, and 1 had peripheral nerve neuropathy.

*Mycoplasma pneumoniae* is a common cause of upper and lower respiratory tract infections; it also affects other organ systems [1]. One of the most common extrapulmonary manifestations is a disorder of the central and/or peripheral nervous system [2]. Various clinical presentations (e.g., meningitis, meningoencephalitis, and transverse myelitis) have already been described elsewhere [3, 4]. In the majority of those cases, the diagnosis of *M. pneumoniae* infection was made on the basis of findings of serological tests [5–8].

We describe 13 patients with various neurological symptoms and signs whose CSF samples yielded *M. pneumoniae* by culture and/or *M. pneumoniae* nucleic acid by PCR. Two cases are presented in detail.

**PATIENTS AND METHODS**

The patients were hospitalized from June 1994 through December 1997 at the University Medical Center (Department of Infectious Diseases or Department of Pediatrics) in Ljubljana, Slovenia. At the onset of illness, they experienced respiratory symptoms, followed by neurological symptoms and signs. For the majority of patients, blood samples were obtained on the first day of hospitalization, and lumbar punctures were performed soon after admission to the hospital. In our study, we included patients for whom blood tests and CSF serological tests for *M. pneumoniae* were requested and for whom blood serological tests indicated acute infection. Almost all serum samples that were obtained on admission to the hospital tested positive for specific IgM antibodies by means of EIA. The aliquots of CSF were frozen and in most cases used later for a retrospective analysis (culture and identification of *M. pneumoniae*; PCR).

*Isolation and identification.* The isolation of my-
coplasmas from the CSF was attempted by use of agar plates and broth media to which 15% horse serum and some other components that support the growth of *M. pneumoniae* were added [9]. The cultures were incubated at 37°C for 3 weeks or until mycoplasma growth was noticed. PCR analysis of some CSF samples yielded positive results for *M. pneumoniae*, but, on culture media, no growth could be detected. Frozen CSF samples were cultured again, and SP-4 media were used to increase the yield of isolates [10]. If there was no growth after 30 days, “blind” subcultures were done on fresh SP-4 medium. Subcultures that failed to yield growth after 1 month were considered negative for *M. pneumoniae*.

Colonies grown on agar plates were identified as *M. pneumoniae* by use of the direct immunofluorescence (DIF) and/or direct immunoperoxidase (DIP) assay and by use of horseradish peroxidase–labeled rabbit IgG to *M. pneumoniae* (type strain FH) and procedures described elsewhere [11]. *M. pneumoniae* type strain FH was obtained from Janet M. Bradbury (University of Liverpool, United Kingdom).

*M. pneumoniae* isolated in broth cultures was identified in 2 ways: (1) by subculture and identification of colonies by use of DIF and DIP, and (2) by Western immunoblotting with use of the horseradish peroxidase–labeled monoclonal antibody (mAb) to the 37-kDa protein (P37), which bears a species–specific epitope (mAb from an *M. pneumoniae* antigen detection kit; Virion-Serion). In addition, mAb 2C2/C3 to the *M. pneumoniae* 60-kDa GroE1 protein and mAb 8285 to P1 adhesin (Chemicon) were used to confirm that isolates contained *M. pneumoniae*.

**PCR.** CSF sample aliquots were centrifuged at 10,000 g for 15 min, and the DNA was extracted from the pellets. Two sets of oligonucleotide primers, MP-P11/MP-P12 and MP5-1/MP5-2, were purchased from MWG-Biotech. The first primer set, MP-P11/MP-P12, enabled amplification of a part of the P1 adhesin gene of *M. pneumoniae* [12], and the second primer set, MP5-1/MP5-2, served for amplification of the *M. pneumoniae*–specific sequence [13]. The standard PCR reaction contained 1.5 μL of the sample lysate, 15 pmol of each primer (MP-P11 and MP-P12, or MP 5-1 and MP5-2), 0.2 nM dNTP (Pharmacia Biotech), 50 mM of KCl, 10 mM of Tris-HCl (pH, 8.3), 1.5 mM of MgCl2, 0.01% (w/v) gelatin, and 0.5 U of Taq polymerase (Life Technologies) in 20 μL of reaction volume. Following denaturation for 5 min at 95°C, 35 cycles were performed (1 min at 95°C, 45 s at 55°C, and 45 s at 72°C). The final elongation step was extended for 2 min at 72°C. The PCR products were analyzed by use of 1.5% agarose gel electrophoresis and ethidium bromide staining. PCR products amplified by primers MP-P11/MP-P12 were sequenced by use of the ABI PRISM 301 automated DNA sequencer (Perkin Elmer) and the Rhodamine Terminator Cycle Sequencing Kit (Perkin Elmer), according to the manufacturer’s instructions.

**RESULTS**

The isolation of *M. pneumoniae* from CSF was attempted for 19 patients, and the results of cultures were found to be positive for 9 patients. Of the CSF samples, 8 had positive results in broth culture; they also yielded growth of *M. pneumoniae* colonies after subculture onto agar plates. Direct seeding of the CSF on agar plates yielded 6 cultures in which *M. pneumoniae* colonies were identified by use of DIF and/or DIP. Thus, 1 CSF sample yielded growth of *M. pneumoniae* only on agar medium (rare colonies), whereas the results of broth culture remained negative.

Results of PCR analysis were positive for all patients with positive culture results. An additional 4 patients had positive PCR results only. Sequences of the P1 genes revealed at least a 98% sequence identity with the reference P1 gene sequence (nucleotides 210–610) and with the corresponding sequence of the FH strain. However, certain distinct sequence variations, which probably were characteristic of the *M. pneumoniae* populations in CSF samples of individual patients, were also found.

There were 6 female and 7 male patients, most of whom were children or young adults. On clinical grounds, the patients were divided into 2 groups. Eight patients (patients 1–8, table 1) who were hospitalized in the Department of Infectious Diseases at the University Medical Center developed acute-onset CNS infection soon after respiratory symptoms appeared. The lumbar puncture was performed by day 13 (range, 2–13 days) after the onset of the illness and no later than the third day after the appearance of neurological signs and symptoms. The erythrocyte sedimentation rate was elevated in 3 patients and the leukocyte count was elevated in 5 patients. The results of lumbar puncture analysis are shown in table 1. Two patients with acute meningitis (patients 7 and 8) had an uncomplicated course of disease and recovered quickly without sequelae. Five patients suffered from acute meningoencephalitis. Two boys with meningoencephalitis (patients 3 and 5) were somnolent; in one (patient 5), lower-limb weakness and hypotonia were noted, with absent reflexes and bilateral Babinski’s sign. Both improved soon after the introduction of therapy with mannitol and furosemide for probable cerebral edema.

In 3 patients (patients 1, 2, and 6), the level of consciousness gradually worsened, resulting in deep coma. Mechanical ventilation was required. The treatment of all 3 patients was practically identical: each received iv erythromycin, acyclovir, a third-generation cephalosporin (cefotaxime or ceftriaxone), mannitol, and furosemide. The outcome for 1 of the 3 patients, a 17-year-old adolescent boy (patient 6), was good; his condition began to improve 4 days after the beginning of treatment, and he recovered completely. The second patient (patient 1) survived with residual mental deficit. The third patient (patient
Table 1. Characteristics of and findings for 13 patients with neurological signs and symptoms of *M. pneumoniae* infection.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Sex</th>
<th>Neurological symptom(s) and/or sign(s)</th>
<th>Lumbar puncture findings</th>
<th>Results of culture, PCR</th>
<th>EEG findings</th>
<th>Cranial CT findings</th>
<th>Antibiotic therapy</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>F</td>
<td>Headache, agitation, coma</td>
<td></td>
<td>+, +</td>
<td>Diffuse</td>
<td>Normal</td>
<td>Em, Acy, Ctri</td>
<td>ME, Pn</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>F</td>
<td>Headache, confusion, paresis of left lower limb, positive Babinski’s sign, coma</td>
<td></td>
<td>+, +</td>
<td>Focal Edema</td>
<td></td>
<td>Em, Acy, Ctax</td>
<td>ME, Pn</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>M</td>
<td>Somnolence</td>
<td></td>
<td>+, +</td>
<td>Focal</td>
<td>Edema</td>
<td>Em</td>
<td>ME, Pn</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>F</td>
<td>Dysarthria left facial nerve palsy, left-side hemiparesis</td>
<td></td>
<td>+, +</td>
<td>Diffuse Infarction</td>
<td></td>
<td>Em, Ctax, Mtz</td>
<td>CVI</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>M</td>
<td>Confusion, right abducens and lower-limb paresis, absent knee and ankle jerk, bilateral Babinski’s sign</td>
<td></td>
<td>+, +</td>
<td>Diffuse Edema</td>
<td></td>
<td>Ctri</td>
<td>ME</td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>M</td>
<td>Tonic-clonic seizures, followed by coma</td>
<td></td>
<td>+, +</td>
<td>Diffuse Hypodense lesion</td>
<td></td>
<td>Em, Acy, Ctri</td>
<td>ME, Pn</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>M</td>
<td>Headache</td>
<td></td>
<td>+, +</td>
<td>ND</td>
<td>ND</td>
<td>Ctax</td>
<td>Meningitis</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>M</td>
<td>Headache</td>
<td></td>
<td>+, +</td>
<td>ND</td>
<td>ND</td>
<td>Azm</td>
<td>Meningitis</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>F</td>
<td>Focal seizures, followed by impairment of consciousness</td>
<td></td>
<td>−, +</td>
<td>Epileptiform Normal</td>
<td>ND</td>
<td>None</td>
<td>Partial epilepsy</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>F</td>
<td>Headache, distraction, forgetfulness, unstable gait</td>
<td></td>
<td>−, +</td>
<td>Normal ND</td>
<td>None</td>
<td>None</td>
<td>Respiratory infection</td>
</tr>
<tr>
<td>11</td>
<td>16</td>
<td>F</td>
<td>Headache, impaired concentration and thinking, blurred vision</td>
<td></td>
<td>−, +</td>
<td>Normal</td>
<td>None</td>
<td>None</td>
<td>Photosensitivity</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>M</td>
<td>Headache, impaired concentration, irritability, paresthesia, blurred vision</td>
<td></td>
<td>−, +</td>
<td>Generalized epilepsy Maxillary sinusitis</td>
<td>None</td>
<td>Edema of optic disk</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>52</td>
<td>M</td>
<td>Lower limb and facial dysesthesia, progressive fatigue, hypersomnia</td>
<td>NA NA</td>
<td>−, +</td>
<td>Mild, diffuse slowing ND</td>
<td>Rox</td>
<td>Demyelinating disease (?)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Acy, acyclovir; Azm, azithromycin; Ctax, cefotaxime; Ctri, ceftriaxone; CVI, cerebrovascular infarction; EEG, electroencephalography; Em, erythromycin; F, female; M, male; ME, meningoencephalitis; Mtz, metronidazole; NA, not available; ND, not done; Pn, pneumonia; Rox, roxithromycin; +, positive; −, negative.
2) died 5 weeks after onset of the disease. The autopsy revealed encephalomalacia, bilateral pneumonia, and splenomegaly.

The neurological symptoms in the second group of 5 patients (patients 9–13; table 1) began gradually and lasted longer before admission to the hospital. Their laboratory data, including CSF examination findings, were normal. An oligoclonal IgG fraction was present in 1 patient (patient 13), for whom this determination was requested on clinical grounds. Three patients were treated with a macrolide antibiotic before the onset of neurological symptoms. Isolation of *M. pneumoniae* was successful for only 1 patient.

**CASE REPORTS**

**Case 1.** A 28-year-old woman was admitted with a 6-day history of high fever and productive cough. She was treated at home with amoxicillin/clavulanic acid. During the night before hospitalization, she suddenly noticed weakness in her left arm and leg, which diminished temporarily but worsened again a few hours later. On admission to the hospital, she was afebrile and had dysarthria, with seventh-nerve paresis and left hemiparesis. Chest examination revealed prolonged expiration and diffuse rales.

Laboratory values (i.e., WBC, RBC, and platelet counts; liver function tests; and creatinine and blood urea nitrogen levels) were within normal limits, with the exception of a mild elevation of the erythrocyte sedimentation rate and C-reactive protein level (22 mm/h and 19 mg/L, respectively). The analysis of blood gases showed low-grade hypoxia. The findings of the chest radiograph were normal. The initial cranial CT scan (with and without contrast) disclosed a hypodense lesion located in the right parietotemporal region and described as a cerebrovascular infarction. After the collection of blood and CSF samples for bacteriologic culture and serologic tests, therapy with antibiotics (cefotaxime, metronidazole, and erythromycin) and diuretics (mannitol and furosemide) was initiated. The findings of electrocardiography, echocardiography, and abdominal ultrasonography were normal. The electroencephalogram showed a lateralized abnormality with high-voltage delta activity over the right hemisphere.

During the subsequent 3 weeks, the patient’s neurological status improved. She was able to speak and walk much better. The bacterial cultures of the CSF fluid remained sterile, with the exception of *M. pneumoniae*; results of serologic tests for various microorganisms (e.g., herpes simplex virus, *Borrelia burgdorferi*, arbovirus B, and *Chlamydia* species) were negative. The antimycoplasmal IgM value was positive, and the IgG value was borderline in the first serum sample. Tests for a possible autoimmune disorder or vasculitis (including antinuclear, extractable nuclear antigen, antineutrophilic, cytoplasmic, and antcardiolipin antibody tests, as well as lupus anticoagulant tests) all yielded negative results. The second cranial CT scan showed shrinkage of the lesion. Cerebral angiography showed an occlusion of the second insular artery. Half a year later, the neurological deficit was minimal.

**Case 2.** A 15-year-old girl had a sore throat and was treated with azithromycin for 3 days. From then on, she experienced daily headaches. From time to time, just before the onset of headache, she would complain of blurred vision, and the headache was accompanied by dizziness. She could not concentrate very well and had difficulty thinking.

One month later, when she was admitted to the hospital to undergo diagnostic procedures, her neurological status was completely normal. Findings of routine laboratory tests of the blood and urine and analysis of the CSF were normal. No abnormalities were found on the CT and MRI brain scans or on ophthalmologic examination. Electroencephalography showed irregular background activity with slow waves in the posterior regions. A mild asymmetry was present inconsistently—slow components were prominent centrally and posteriorly on the right side, and sharp waves were prominent on the left side. Intermittent photostimulation provoked generalized bursts of spike-wave activity between 8 and 28 Hz that were accompanied by twitching of the head, eyelids, and hands.

Treatment with sodium valproate was initiated. Electroencephalography (performed 1 month later) revealed fewer epileptiform discharges, but the background activity was slightly more irregular. Intermittent photostimulation provoked diffuse discharges of sharp and slow waves, which were no longer expressed clinically. She did not appear for follow-up examination.

**DISCUSSION**

*M. pneumoniae* infection is generally thought to cause a relatively mild clinical presentation. In one study, 27 (4.8%) of 560 patients with mycoplasmal infection developed symptoms and signs of peripheral and/or CNS disorders [3]. The course of the disease was severe in some cases, especially in patients with meningoencephalitis. According to the study that involved the largest published series of patients, neurological complications appear in 0.01% of patients with *M. pneumoniae* infection [4]. In children, the most common manifestation was meningoencephalitis, whereas more varied clinical presentations were noted in adult patients. The fatality rate of neurological patients was 8% [4].

In the majority of cases that have been published, diagnosis has relied on commercially available serologic tests, such as CF, ELISA, or cold agglutinins. Reported cases that were confirmed by the isolation of *M. pneumoniae* from the CSF [14, 15] or by detection by use of PCR [16–19] are not common.

In our study, the attempt to cultivate *M. pneumoniae* from
CSF and to proceed with PCR was made only for those patients in whom respiratory symptoms were combined with neurological symptoms and in whom serum antibodies to *M. pneumoniae* (IgM and/or IgG, per ELISA) were present. During the 4-year period, 9 of 19 patients selected in this manner had positive culture results and 13 had positive results of PCR analysis. The identity of PCR fragments amplified with MP-P11/MP-P12 primers was confirmed by means of DNA sequencing. The clinical presentations did not differ from those already described. Isolation was successful for patients with a recent history of infection, but only mycoplasmal nucleic acids were detected if a longer period of time had elapsed between the onset of the disease and lumbar puncture.

Four patients with meningoencephalitis were treated with erythromycin, including 1 who died. The deceased patient began receiving antibiotic therapy with erythromycin 1 week after the onset of a febrile illness with respiratory symptoms and 1 day after becoming confused. Three patients received a macrolide orally before neurological complications began. Macrolides and tetracyclines are considered effective for respiratory illness with respiratory symptoms and 1 week after becoming confused. Three patients received a macrolide receiving antibiotic therapy with erythromycin 1 week after the onset of a febrile illness with respiratory symptoms and 1 day after becoming confused. Three patients received a macrolide orally before neurological complications began. Macrolides and tetracyclines are considered effective for respiratory tract infection caused by *M. pneumoniae*, but their role in the treatment of neurological complications is less clear. There are very limited data on erythromycin concentrations achieved in the CSF of patients with meningitis, even after large parenteral doses. In the CSF, tetracycline reaches 10%–26% of the serum level [20].

The mechanism of damage caused by *M. pneumoniae* remains unclear [4]. The isolation of *M. pneumoniae* from the CSF undoubtedly confirms the invasion of the CNS or, at least, of the CSF. Two other mycoplasmas, *M. neurolyticum* and *M. gallisepticum*, produce a neurotoxin, but no toxin production in *M. pneumoniae* has been reported. Circulating immune complexes were detected in the serum samples of some patients, so an autoimmune process is believed to be induced by *M. pneumoniae* infection. In this case, immunosuppressive therapy may prove to be more beneficial than therapy with antibiotics, especially because macrolides and tetracycline achieve only low concentrations in the CSF.

References