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ORIGINAL ARTICLE

Prognostic values of a combination of intervals between respiratory illness and onset of neurological symptoms and elevated serum IgM titers in *Mycoplasma pneumoniae* encephalopathy

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M. pneumoniae-associated encephalopathy;
Mycoplasma pneumonia;
Polymerase chain reaction (PCR)

Background/Purpose: To retrospectively analyze the clinical manifestations of *Mycoplasma pneumoniae* (*M. pneumoniae*)-associated encephalopathy in pediatric patients.
Methods: Pediatric patients with positive serum anti-*M. pneumoniae* immunoglobulin M (IgM) were enrolled in this study. Clinical signs and symptoms, laboratory data, neuroimaging findings, and electrophysiological data were reviewed.
Results: Of 1000 patients identified, 11 (1.1%; male:female ratio = 7:4) had encephalopathy and were admitted to the pediatric intensive care unit. Clinical presentation included fever, symptoms of respiratory illness, and gastrointestinal upset. Neurological symptoms included altered consciousness, seizures, coma, focal neurological signs, and personality change. Neuroimaging and electroencephalographic findings were non-specific. Specimens of cerebrospinal fluid (CSF) for *M. pneumoniae* polymerase chain reaction (PCR) were negative. Higher *M. pneumoniae* IgM titers and longer intervals between respiratory and CNS manifestations were associated with worse outcomes.

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Conclusion: Clinical manifestations of *M. pneumoniae*-associated encephalopathy were variable. Diagnosis of *M. pneumoniae* encephalopathy should not rely on CSF detection of *M. pneumoniae* by PCR. *M. pneumoniae* IgM titers and intervals between respiratory and CNS manifestations might be possibly related to the prognosis of patients with *M. pneumoniae*-associated encephalopathy.
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Introduction

Encephalitis refers to an acute, usually diffuse, inflammatory process affecting the brain.[1] The incidence is approximately 1.5—13.8 cases per 100,000.[2,3] Encephalitis can be caused by a variety of afflictions, most often by a virus, but occasionally by bacteria or other pathogens. Encephalopathy is a brain disease, damage, or malfunction. The causes of encephalopathy are numerous and varied. The majority of cases arise from infection, liver damage, anoxia, or kidney failure. Whether known or unknown infections cause encephalitis, leading to infection-associated encephalopathy, remains unclear.[4]

A series of microbes can cause acute encephalopathy, but agents, in most cases, remain unknown. *Mycoplasma pneumoniae* is a common pathogen of the respiratory system in school-aged children and young adults.[5] The prevalence of *M. pneumoniae* was 10% in 2010, 17% in 2011, and 15% in 2012, supporting a trend of cyclic endemics every 3～5 years for *M. pneumoniae* infections in Taiwan.[6] This microorganism can affect blood, skin, joints, central nervous system (CNS), liver, pancreas, and the cardiovascular system, causing extrapulmonary manifestations.[7,8] *M. pneumoniae*-associated neurologic illness is not rare and is identified in 1% of encephalopathy cases.[9] Over the years, several case reports have described a wide variety of complications associated with *M. pneumoniae* infection, including cases with neurologic complications in the absence of systemic symptoms and cases that appear to be post-infectious, rather than being caused directly by the organism.[10,11] Despite several cases being reported for many years, the extent to which *M. pneumoniae* is involved in the causation of human neurologic disease is not yet known.

Several laboratory methods can detect *M. pneumoniae* infection, including isolation, complement fixation, serology, and molecular assays. However, each of these methods has limitations. Isolation of *M. pneumoniae* is inconvenient, time-consuming, and causes the generation of inconsistent results.[12] Although direct detection of *M. pneumoniae* from brain tissue and/or cerebrospinal fluid (CSF) supports the belief that *M. pneumoniae* is a major cause of encephalopathy, the positive rate of detection of *M. pneumoniae* from CSF specimens by polymerase chain reaction (PCR) is variable and low.[13] Commercially available serologic test kits for the detection of antibodies to *M. pneumoniae*, possess inherent limitations of specificity and sensitivity. The test relies on patient compliance with the timely acquisition of acute- and convalescent-phase serum samples for accurate interpretation. However, the serology testing for the diagnosis of *M. pneumoniae* is imprecise, because patients with neurologic involvement due to other agents may sometimes develop elevated antibody titers to *M. pneumoniae*.[5] Despite these drawbacks, serology is still a sensitive test for detecting acute *M. pneumoniae* infection in pediatric patients in contrast with adult patients.[8] Furthermore, several papers continue to suggest that the immunoglobulin M (IgM) test is a readily convenient method to assist in the diagnosis of *M. pneumoniae*-associated encephalopathy.[8,10—12]

Neurological sequelae in *M. pneumoniae*-associated CNS illness are very high. A mounting number of reports show neurological sequelae in 48—64% of cases of *M. pneumoniae*-associated encephalopathy.[14] Despite the fact that therapies including antibiotics, intravenous immunoglobulin (IVIG), and steroids have been proposed by several articles, the role of these treatments in *M. pneumoniae*-associated encephalopathy remains unclear, because the benefits of these treatments lack the comparison of control groups in clinical trials and spontaneous recovery in some cases without any treatment has been reported.[5,8,14] Therefore, because there is no strong evidence to support these treatments, it is worth exploring the related prognostic factors for *M. pneumoniae*-associated encephalopathy.

Narita et al[15] reported that "intervals between respiratory and CNS manifestations" may be pivotal in understanding the mechanism of *M. pneumoniae*-associated encephalopathy. They proposed that if the interval is $<$7 days, the mechanism for *M. pneumoniae* is direct invasion. Adversely, if the intervals are $>$7 days, the mechanism is immune-mediated. However, if the interval is a key point in approaching the mechanisms of *M. pneumoniae*-associated encephalopathy, a retrospective analysis of medical records may verify the connection between the mechanisms of this disease and the intervals. Moreover, whether these connections relate to the outcomes of patients with *M. pneumoniae*-associated encephalopathy, are worthy of further investigation. We therefore conducted a retrospective review of charts targeting *M. pneumoniae*-associated encephalopathy and analyzed their outcomes after discharge for $\geq$6 months.

Methods

Selection criteria

From January 1, 2003 to December 31, 2010 patients were selected for inclusion in this study if they fulfilled the following criteria: (1) $<$18 years of age; (2) positive for *M.*

pneumoniae by serology within 24 hours of acute onset of neurologic symptoms; and (3) any new-onset of acute encephalopathy symptoms, including confusion, disorientation, coma, or inability to talk, new onset of seizures, focal neurological signs (tremor, rigidity, dysarthria, acute urine retention, decreased muscular power, or decreased deep tendon reflex), and personality or behavioral change.

Instead, patients with a history of neurological illness, such as meningitis, seizure/epilepsy, family history of seizure/epilepsy, progressive neurological disorder, electrolyte imbalance, or co-infection, were excluded. All information was collected by retrospective chart review regarding age, sex, clinical symptoms, electroencephalography, neuroimaging, and clinical laboratory tests, including complete blood count, C-reactive protein (CRP), and CSF analysis. This study was approved by the local institutional review board (Tri-Service General Hospital Institutional Review Board approval number 100-05-130).

IgM for the detection of *M. pneumoniae*

IgM-capture enzyme-linked immunosorbent assay (ELISA; Sero *M. pneumoniae* Kit, Savyon Diagnostics Ltd., Ashdod, Israel) were used for *M. pneumoniae* detection. The generated microplate-based IgM-ELISA results were considered positive when more than 20 BU/mL of specific antibodies to *M. pneumoniae* per milliliter serum were present. We rechecked the datum 2 weeks later if the initial value was within 10∼20 BU/ml, and considered it was positive if the result of second serum sample was more than 20 BU/ml, and negative if the result was less than 20 BU/ml.

PCR for the detection of *M. pneumoniae*

CSF samples were obtained within 1−2 days following admission, refrigerated at 4°C for <72 hours, and then frozen at −70°C before PCR assay. The DNA extraction and PCR amplification protocol were according to the method of Lin et al.[16]

Other laboratory investigations

Blood and CSF samples from all these cases underwent bacterial cultures, viral isolation, serology tests, and PCR to identify possible pathogens such as Epstein-Barr virus (EBV), herpes simplex virus (HSV), varicella-zoster virus (VZV), and enterovirus, and the detection of Influenza A/B antigen.

Outcomes

Patients' outcomes were determined based on evaluation records of the clinic visit 6 months or more after the encephalopathy episode. For purposes of data analysis, outcomes were divided into "good" or "poor" according to the patients' 6-month follow-up. A "good response" indicated that the patient had fully recovered and all neurological functions had returned to baseline, whereas a "poor response" indicated that the patient still had neurological or psychological symptoms that were not recorded before admission.

Statistical data

Statistical analysis was performed using SPSS 18.0 (SPSS, Inc., Chicago, IL, USA). Descriptive statistics and quantitative data are expressed as mean ± standard deviation and percentages. Fishers' exact test was used in the comparison of clinical neurological manifestations, serum IgM level, and intervals between respiratory illness and the onset of neurological symptoms. The prognostic values of IgM titers and intervals were analyzed using the receiver operating characteristic (ROC) curve and established optimal cutoffs corresponding to the highest differential positive rate: [sensitivity − (1 − specificity)]. Statistical significance was set at $\alpha = 0.05$, $p < 0.05$.

Results

Of 1000 pediatric patients (male:female ratio = 549:451) with positive anti-*M. pneumoniae* IgM, 11 children (1.1%; male:female ratio = 7:4) presented with encephalopathy and were admitted to the pediatric intensive care unit. Their ages ranged from 3 years to 17 years (mean = 8 ± 4.2 years). Presenting features included fever (10/11), respiratory illness (11/11), gastrointestinal symptoms (3/11), altered level of consciousness (8/11), coma (3/11), generalized tonic-clonic seizures (4/11), focal neurological signs (6/11), and personality or behavioral change (3/11; Table 1). Chest radiographs revealed perihilar infiltration without pneumonia (11/11). The average intervals between respiratory illness and onset of neurological symptoms were 9.2 ± 4.5 days (range = 1−14 days; Table 1).

The patients' white blood cell (WBC) counts, platelet counts, and glucose levels were within the normal range, but serum CRP levels were mildly elevated (0.7 ± 0.7 mg/dL). In addition, biochemistry and cell count studies of CSF were within the normal range, except that the WBC count showed mild pleocytosis (28.6 ± 71.3/mm^3). The CSF PCR for detection of *M. pneumoniae* was negative (Table 1). Electroencephalography (EEG; $n = 11$) revealed diffuse slowing activity (7/11), epileptiform discharge (4/11), and normal findings (2/11; Table 1). Cranial computed tomography (CT) scans ($n = 7$) were performed on admission, and no leptomeningeal enhancement or brain swelling was noted. Brain MRI ($n = 8$), which was performed during hospitalization from 2 days to 1 week, revealed a diffuse high-intensity signal in the cortex (1/8), meningeal enhancement (3/8), decreased N-acetyl aspartate/creatine (NAA) ratio (2/8), unilateral ventricle dilatation (1/8), and normal findings (2/8; Table 1).

Eight patients (72.7%) received a 3-day course of a macrolide (azithromycin 10 mg/kg). Case 2 received methylprednisolone and IVIG, because of rapid deterioration of neurological function. Cases 6 and 9 received azithromycin and IVIG in an attempt to accelerate recovery. Cases 1 and 4 did not receive azithromycin, corticosteroids, or IVIG, due to a delay in diagnosis and only Case 1 recovered completely without neurological sequelae.

Sequelae at 6 months or more for follow-up included focal seizures (2/11), mental impairment (2/11), muscle rigidity and joint contracture (1/11), and decreased muscle power (1/11). Five patients fully recovered and six showed

Table 1 Clinical symptoms, laboratory data, brain electrophysiological profile, brain images, treatment, and outcome

Case	Sex	Age (y)	Prodrome	CNS manifestation	Intervals between respiratory and CNS manifestations (d)	Serum			CSF					EEG	CT	MRI	Treatment	Sequelae	Outcome at 6-mo follow-up
						WBC (no./μL)	CRP (mg/dL)	IgM (BU/mL)	WBC (no./μL)	RBC (no./μL)	TP (mg/dL)	Glucose (mg/dL)	PCR						
1	M	13	RI, N/V	A, F	5	3930	0.40	39.90	240	0	43	61	Negative	Normal	Normal	ND	None	Normal	Good
2	M	7	Fever, RI	A, F	11	8100	0.19	73.42	6	14	24	61	Negative	MFE	ND	DN	Methylprednisolone IVIG	MJ	Poor
3	M	5	Fever, RI	A	1	17,320	2.63	10.20	3	9	14	62	Negative	SW	Normal	ME	Azithromycin	Normal	Good
4	M	10	Fever, RI	A, P	14	4900	0.03	62.70	0	4	20	81	Negative	SW	ND	Normal	None	MI	Poor
5	F	4	Fever, RI, N/V	A, P	4	4310	0.50	20.10	0	2	24	68	Negative	SW	Normal	ND	Azithromycin	Normal	Good
6	M	6	Fever, RI	C, S	12	19,520	0.99	109.20	0	2	28	76	Negative	MFE SW	ND	DS	Azithromycin IVIG	FS	Poor
7	M	3	Fever, RI, N/V	C, F, S	14	8900	0.54	87.00	0	2	20	74	Negative	SW	Normal	ME	Azithromycin	DP	Poor
8	M	17	Fever, RI	A	10	8300	0.60	152.00	0	6	21	63	Negative	SW	Normal	DV	Azithromycin	MI	Poor
9	F	10	Fever, RI	A, F	7	3900	0.78	21.90	0	4	14	55	Negative	Normal	ND	Normal	Azithromycin IVIG	Normal	Good
10	F	7	Fever, RI	A, F, P, S	9	1200	0.65	26.60	30	18	69	84	Negative	MFE SW	Normal	ME DN	Azithromycin	Normal	Good
11	F	6	Fever, RI	C, F, S	14	17,700	0.39	154.60	35	60	94	83	Negative	GE	Normal	ND	Azithromycin	FS	Poor
Mean ± SD		8 ± 4.2			9.2 ± 4.5	9734.6 ± 5855.9	0.7 ± 0.7	68.9 ± 51.9	28.6 ± 71.3	11.0 ± 17.2	33.7 ± 25.5	69.8 ± 10.2							

A = altered consciousness; BU = binding units; C = coma; CRP = C-reactive protein; CSF = cerebrospinal fluid; CT = computed tomography; DN = decreased NAA peak; DP = decreased muscle power; DS = high diffuse signal in cortex; DV = dilatation of right ventricle; EEG = electroencephalography; F = focal neurological signs (tremor, rigidity over limbs, dysarthria, acute urine retention, decreased muscular power, decreased deep tendon reflex); FS = focal seizures; GE = generalized epileptiform discharge; IgM = immunoglobulin M; IVIG = intravenous immunoglobulin; ME = meningeal enhancement; MFE = multifocal epileptiform discharge; MI = mental impairment; MJ = muscle rigidity and joint contracture; MRI = magnetic resonance imaging; ND = not done; N/V = nausea or vomiting; P = personality and behavioral change; PCR = polymerase chain reaction for detection of *Mycoplasma pneumoniae*; RBC = red blood cell count; RI = respiratory illness; S = seizure; SW = slow wave; TP = total protein; WBC = white blood cell count.

poor outcome after 6 months or more at their most recent clinical visit (Table 1). We evaluated the clinical neurological manifestations, serum IgM level, and intervals between respiratory illness and onset of neurological symptoms between the good and the poor outcome groups. We found coma ($p = 0.03$), IgM level >60 BU/mL ($p = 0.008$), and intervals between respiratory illness and onset of neurological symptoms >10 ($p = 0.008$) all in the poor outcome group (Fig. 1). The optimal cutoff calculated in our series of patients was 9.5 days for the intervals between respiratory illness and onset of neurological symptoms and 51.3 BU/mL for IgM levels by the ROC curve.

Discussion

Although fever and respiratory tract symptoms were common in our cases and others,[8] the clinical signs and symptoms caused by *M. pneumoniae*, including diverse manifestations, CSF abnormalities, and findings on EEG and neuroimaging, were indistinguishable from other pathogens. Hence, an accurate laboratory diagnosis of *M. pneumoniae* infection is vital. Although several reports showed positive results in the detection of *M. pneumoniae* in CSF specimens from patients with *M. pneumoniae*-associated encephalopathy,[5,7,8,13–16] in this study, technical difficulties, the possible existence of inhibitors,[17] an unfavorable environment for *M. pneumoniae* growth in CSF, antibiotic treatment prior to the collection of specimens,[18] immune responses, and other undetermined factors might explain the negative results of PCR in the CSF in our cases and others.[19] Importantly, a negative result of *M. pneumoniae* DNA in the CSF cannot exonerate the involvement of *M. pneumoniae* in CNS. *M. pneumoniae* culture is not recommended for the diagnosis of acute *M. pneumoniae* infection, because *M. pneumoniae* requires special media and needs weeks to grow.[20] The diagnosis can also be made by serologic tests for IgM and IgG antibodies to *M. pneumoniae* in paired (acute- and convalescent-phase) serum samples. For the IgG study, it may take at least 3 weeks for the significant rise of IgG titers in the acute infection.[21] For the IgM study, it is fast and convenient and the changes of titers are fairly consistent in childhood during the acute phase.[8,22] For these reasons, the *M. pneumoniae* IgM test is commonly used in the screening of pediatric patients with suspicious *M. pneumoniae* infection.

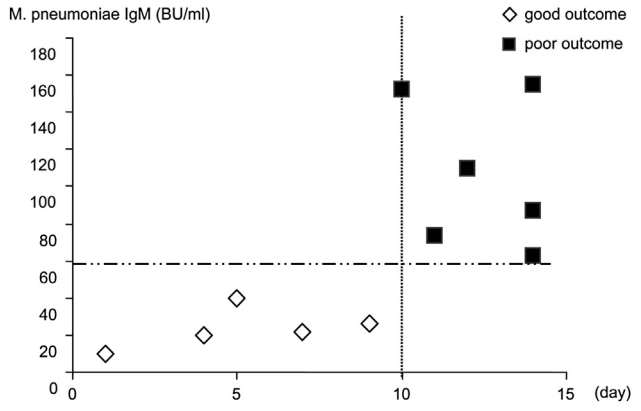

Figure 1. Correlation between the level of immunoglobulin M and intervals between respiratory illness and onset of neurological symptoms. ◇, patients with good outcome; ■, patients with poor outcome. BU/mL = binding units per milliliter serum; IgM = immunoglobulin M. The vertical broken line is the cutoff date of the intervals between respiratory illness and onset of neurological symptoms between groups with good outcomes and poor outcomes; the horizontal broken line is the cutoff IgM concentration between groups with good outcomes and poor outcomes.

Because the etiology of the encephalopathy was initially unclear, we performed a wide range of bacteriological and viral tests. Apart from a positive *M. pneumoniae* IgM, most of the tests were negative. Whether *M. pneumoniae* is playing a role as the main cause or a "bystander" and/or a "mixed infection" remains unclear.[23] It has been recently reported that co-infection of *M. pneumoniae* with other bacterial and/or viral pathogens is not rare.[24,25] We cannot exclude the possibility that some children might have co-infection with other bacterial or viral pathogens, such as Japanese encephalitis (JE) virus, West Nile virus, or Hendra/Nipah virus, which are more virulent than *M. pneumoniae*. Additionally, several viral ant*igens may cross-react with the M. pneumoniae IgM assay and cause* false-positives. *As such, the clinical implications of mixed infections, compared with a sole agent, remain unresolved. These are the limitations of this study.*

The incidence of *M. pneumoniae*-associated encephalopathy in this study was 1.1%, which is consistent with data from previous studies.[26] More than half of the patients (6/11) in this study showed neurological sequelae after 6 months or more follow-up. Because little evidence supported macrolides and immunomodulatory agents, such as corticosteroids and IVIG, for *M. pneumoniae* associated encephalopathy, it would be useful to have some parameters to predict their outcomes. As some clinical characteristics of *M. pneumoniae* are similar to those observed in systemic viral infections, the titers of *M. pneumoniae* IgM, like the titers of Cytomegalovirus, might link to patients' outcomes[27]. Additionally, the intervals between respiratory and CNS manifestations were proposed to be related to the mechanisms and outcomes of patients with *M. pneumoniae* associated encephalopathy.[15] However, we think that each of them is too simplified and shallow in the understanding of this disease. A combination of these two viewpoints in our cases led to an important conclusion, that is, higher *M. pneumoniae* IgM titers and longer intervals between respiratory and CNS manifestations are associated with worse outcomes. We postulated that in patients with relatively low concentrations of IgM titers (<51.3 BU/mL) and shorter intervals (<9.5 days), the IgM, which was induced/activated by *M. pneumoniae* or other unidentified pathogens, might not cause too many dysregulated immune cascades and less tissue damage and sequelae. However, if the intervals were too long and the titers of IgM were too high, the consequences of induced/activated direct and/or indirect immune attacks might end in a poor prognosis. These findings suggest that IgM titers and the intervals between respiratory illness and onset of neurological symptoms may be associated with a worse outcome in patients with *M. pneumoniae* encephalopathy.

In conclusion, patients with *M. pneumoniae*-associated encephalopathy may show a wide array of manifestations. A negative result of *M. pneumoniae* DNA in the CSF cannot exonerate the involvement of *M. pneumoniae* in the CNS diseases. A combination of the intervals between respiratory illness and onset of neurological symptoms and serum IgM titer may connect to patients' outcomes.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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