AQP4 antibody–positive Thai cases

Clinical features and diagnostic problems

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ABSTRACT

Objective: To evaluate the prevalence of aquaporin-4 (AQP4) antibody in Thai patients with idiopathic inflammatory demyelinating CNS diseases (IIDCDs) and to analyze the significance of the autoantibody to distinguish neuromyelitis optica (NMO) and other NMO spectrum disorders (NMOSDs) from other IIDCDs, especially multiple sclerosis (MS).

Methods: We retrospectively evaluated 135 consecutive patients with IIDCDs seen at the MS clinic at Siriraj Hospital, Bangkok, Thailand, and classified them into NMO, NMOSDs, optic-spinal MS (OSMS), classic MS (CMS), and clinically isolated syndrome (CIS) groups in this order with accepted diagnostic criteria. The patients' coded sera were tested separately for AQP4 antibody. Then the relations between the clinical diagnosis and the AQP4 antibody serologic status were analyzed.

Results: Among the 135 patients, 53 (39.3%) were AQP4 antibody–positive. Although the AQP4 antibody–positive group had features of NMO, such as female predominance, long cord lesions (>3 vertebral bodies), and CSF pleocytosis, only 18 patients (33% of 54) fully met Wingerchuk 2006 criteria except for AQP4 antibody–seropositive status. We also detected some AQP4 antibody–positive patients in the OSMS (4 of 7), CMS (11 of 46), and CIS (1 of 16) groups. These patients had been misdiagnosed with MS because they often had brain lesions and never underwent spinal cord MRI examination or lacked long cord lesions.

Conclusions: AQP4 antibody was highly prevalent (almost 40%) in Thai patients with IIDCDs. Moreover, only one-third of AQP4 antibody–positive patients fully met Wingerchuk 2006 criteria, and many were misdiagnosed with MS. A sensitive AQP4 antibody assay is required in this region because the therapy for NMO is different from that for MS. *Neurology*® 2011;77:827–834

GLOSSARY

ANA = antinuclear antibody; **AQP4** = aquaporin-4; **CIS** = clinically isolated syndrome; **CMS** = classic multiple sclerosis; **IIDCD** = idiopathic inflammatory demyelinating CNS disease; **IgG** = immunoglobulin G; **MS** = multiple sclerosis; **NMO** = neuromyelitis optica; **NMOSD** = neuromyelitis optica spectrum disorder; **OB** = oligoclonal immunoglobulin G band; **ONMOSD** = other neuromyelitis optica spectrum disorder; **OSMS** = optic-spinal multiple sclerosis; **VB** = vertebral body.

Neuromyelitis optica (NMO) is an idiopathic inflammatory demyelinating CNS disease (IIDCD) that typically develops into severe optic neuritis and longitudinally extensive transverse myelitis.^{1–3} The relation between NMO and multiple sclerosis (MS) has been controversial, but since the discovery of aquaporin-4 (AQP4), an NMO-specific autoantibody,^{4,5} clinical and laboratory features that distinguish NMO from MS have become clear.^{4–6} In addition, treatment strategies for the 2 diseases are different.^{7,8} AQP4 antibody studies showed that besides definite NMO defined by Wingerchuk 2006 criteria,⁹ there are other AQP4 antibody–positive patients with NMO spectrum disorders (NMOSDs).^{10–13}

The ratios of NMO to MS are much higher in Asian countries than in Western countries.^{14,15} Japanese reports showed that approximately 20% of patients with IIDCDs have NMO.¹⁵ The ratio of NMO to MS in southeastern Asia appears even higher,¹⁶ and one might

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CME

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misdiagnose some AQP4 antibody–positive patients with MS and treat them as such. Thus, we expect that detailed analyses with AQP4 antibody tests in a large number of patients with IIDCDs would have therapeutic implications, but there have been no such studies in any southeast Asian country.

The objectives of this study were to analyze the relation between clinical diagnosis and AQP4 antibody serologic status in Thai patients with IIDCDs by applying accepted diagnostic criteria and a sensitive AQP4 antibody assay.

METHODS Patients and study design. A total of 141 consecutive Thai patients with suspected IIDCD visiting the MS clinic at Siriraj Hospital, Mahidol University, Bangkok, Thailand, during the period from May 1, 2009, to February 28, 2010, participated in the study. We made a clinical diagnosis in each patient with the use of the diagnostic criteria and process described below.

Separately from the clinical diagnoses, 2 of us (S.S. and N.P.) collected serum samples of the patients, coded them, and sent them to the laboratory at the Department of Neurology, Tohoku University Graduate School of Medicine, Sendai, Japan, for AQP4 antibody testing. Hence one of us (T.T.) did the AQP4 antibody assay without knowledge of the clinical diagnoses. Then, after we determined the AQP4 antibody serologic status of each patient, we analyzed the relation between the serologic status and the clinical diagnosis.

Standard protocol approvals, registration, and patient consents. The study received approval from an institutional review board/ethics committee. All participants gave written informed consent.

Diagnostic criteria and process of clinical diagnosis. Two neurologists (S.S. and N.P.) reviewed the medical records of the 141 patients with suspected IIDCDs and made a clinical diagnosis in each patient with diagnostic criteria. The neurologists were blinded to each other's decision and reached consensus if a discrepancy occurred. The diagnostic criteria and the process of diagnosis were as follows.

1. NMO: first, we studied whether the patients met Wingerchuk 2006 criteria,⁹ with the exception of AQP4 antibody status. We diagnosed those who fulfilled all of the following 4 criteria with NMO: optic neuritis, acute myelitis, contiguous spinal cord MRI lesion extending over >3 vertebral bodies (VBs), and onset brain MRI not meeting Paty criteria for MS.¹⁷ We further evaluated patients who were “not NMO” in this step in step 2.
2. Other NMO spectrum disorders (ONMOSDs): we diagnosed patients who fulfilled any one of the following 3 criteria with ONMOSD: recurrent optic neuritis without brain lesions, acute myelitis with long spinal cord lesion (>3 VBs) (with or without brain lesions), or optic neuritis or myelitis without long spinal cord lesion (>3 VBs) with brain MRI findings compatible with those seen in NMO, such as symmetric diffuse white matter lesions, symmetric diencephalic lesions, and periaqueductal lesions.^{1,12,18–20} We further evaluated patients who were “not ONMOSD” in this step in step 3.

3. Optic-spinal MS (OSMS): we diagnosed patients who had optic neuritis and acute myelitis with spinal cord MRI lesion not extending over >3 VBs and with normal brain MRI with OSMS. We further evaluated patients who were “not OSMS” in this step in step 4.
4. Classic MS (CMS): we diagnosed patients who fulfilled McDonald 2005 criteria²¹ with CMS. We further evaluated patients who were “not CMS” in this step in step 5.
5. Clinically isolated syndrome (CIS): we diagnosed patients with the first neurologic event caused by inflammatory demyelination who did not meet the brain MRI findings of McDonald 2005 criteria for dissemination in time with CIS.²² We diagnosed patients who were “not CIS” in this step as not having an IIDCD.

AQP4 antibody testing. We incubated human AQP4-transfected human embryonic kidney 293 cells with diluted serum and washed and incubated them with fluorescein-conjugated goat antihuman immunoglobulin G (IgG). We photographed fixed cells using confocal microscopy and scored antibody seropositivity through comparison with mock-transfected cells that did not express AQP4. We performed assays as described previously.²³ We coded all samples at the time of the assays.

Statistical analysis. We used SPSS version 14.0 (SPSS, Chicago, IL) to perform the statistical analysis. We applied Student *t* test for quantitative data and χ^2 and Fisher exact tests for qualitative data. We considered $p < 0.05$ as statistically significant.

RESULTS AQP4 antibody seropositivity in total patients enrolled and in each patient group. Of the 141 Thai patients, 5 were later proved in the clinic to have other diseases (one each with neuro-Behçet disease, stroke, pachymeningitis, myasthenia gravis, and antiphospholipid syndrome), and one patient presenting with unilateral optic neuritis was lost to follow-up. These patients were excluded from the analysis (figure 1). Among the remaining 135 patients with IIDCDs, 53 (39.3%) were seropositive for AQP4 antibody and the remaining 82 were seronegative. AQP4 antibody positivity was seen in OSMS, CMS, and CIS as well as in NMO and ONMOSD (figure 1).

Of the 19 AQP4 antibody–positive patients with ONMOSDs, we diagnosed 2 with Sjögren syndrome.²⁴ Both patients showed spinal cord MRI lesions extending over 7 VBs before developing sialoadenitis with parotid gland enlargement. Tests for SSA and SSB antibodies were positive, and the parotid gland biopsy revealed histopathology consistent with Sjögren syndrome.

Comparison of clinical, MRI, and laboratory findings in AQP4 antibody–positive patients and AQP4 antibody–negative patients (excluding those with inadequate data and other diseases). AQP4 antibody–positive patients included a higher percentage of women, had higher Expanded Disability Status Scale scores,²⁵ and had

Figure 1 Clinical diagnostic process and aquaporin-4 (AQP4) antibody seropositivity in the present study

We examined whether individual patients fulfilled the diagnostic criteria for neuromyelitis optica (NMO), other neuromyelitis optica spectrum disorders (ONMOSD), opticospinal multiple sclerosis (OSMS), classic multiple sclerosis (CMS), and clinically isolated syndrome (CIS) described in the Diagnostic criteria and process of clinical diagnosis section in this order. When a patient did not fulfill the diagnostic criteria for one disease, that disease was excluded, and the next diagnostic criteria were applied until certain criteria were met.

more acute attacks than AQP4 antibody-negative patients. The first clinical manifestations were similar in the 2 groups (table 1).

There was no difference in the percentages of patients who fulfilled the brain MRI findings for McDonald 2005 criteria in the 2 groups. Spinal cord MRI demonstrated a higher rate of long spinal cord lesions (>3 VBs) in the AQP4 antibody-positive group. Cord lesions in the AQP4 antibody-positive group were longer than those in the AQP4 antibody-negative group. AQP4 antibody-positive patients had slightly more cord lesions than seronegative patients.

CSF analysis showed higher numbers of white blood cells in the CSF in the seropositive group than in the seronegative group (53.7 ± 205.8 vs 19.8 ± 57.1 cells/ μ L). The percentage of patients with positive test results for CSF oligoclonal IgG bands (OBs) was similar in the 2 groups. Serologic autoimmune screening tests including antinuclear antibody (ANA), anti-double-stranded DNA, anti-thyroglobulin, and perinuclear antineutrophil cyto-

plasmic antibody did not show any significant differences in the 2 groups.

Clinical features of AQP4 antibody-negative CMS. We compared 35 patients with AQP4 antibody-negative CMS with 18 patients with AQP4 antibody-positive NMO. The group with AQP4 antibody-negative CMS had less female preponderance (71.4% vs 100%) and fewer (0.7 ± 0.7 vs 1.5 ± 0.8) and shorter (1.1 ± 1.0 vs 6.4 ± 3.3 VBs) cord lesions than the group with AQP4 antibody-positive NMO. Of the 35 patients with AQP4 antibody-negative CMS, CSF samples were available for 29 patients and 17 (58.6%) were OB-positive by the isoelectric focusing method; this frequency of OB was much higher than the 20% in patients with AQP4 antibody-positive NMO.

Clinical characteristics of patients who were diagnosed with CMS by the diagnostic criteria but were AQP4 antibody-positive. We judged 11 (20.4%) of the 54 AQP4 antibody-positive patients as not NMO because they did not fully meet Wingerchuk 2006 criteria. We then diagnosed them with CMS in the present diagnostic process. All but one were women, and the onset age ranged from 16 to 43 years (table 2, figure 2).

Of these patients, patient 1 had initial symptoms of unilateral optic neuritis with brain MRI abnormalities compatible with IIDCD and a medium-length spinal cord MRI lesion (2 VBs). She experienced a subsequent attack of bilateral optic neuritis with poor visual recovery ($<20/200$). Patient 2 experienced intractable hiccup/vomiting lasting for 3 weeks. She had brain MRI lesions compatible with McDonald 2005 criteria but had never undergone spinal cord MRI. Patient 3 had bilateral optic neuritis, and brainstem and spinal cord symptoms. Her spinal cord MRI was inaccessible. Follow-up brain MRI out of the time of the study showed a lesion near the fourth ventricle. Patient 4 had an attack of bilateral optic neuritis followed by a myelitis attack, and brain MRI findings compatible with IIDCD. However, her spinal cord MRI was inaccessible. Patient 5 had recurrent neurologic attacks and was OB-positive. She had never had spinal cord MRI. Patient 6 had a recurrent neurologic deficit and was OB-positive. She had spinal cord MRI more than 30 days after the onset. Follow-up brain MRI showed a periaqueductal lesion. Patient 7 had optic neuritis with poor visual recovery ($<20/200$) and brainstem dysfunction and was OB-negative. Her spinal cord MRI was performed later than 30 days after the onset. Patient 8 had recurrent attacks including one attack with paraplegia, sensory impairment, and bowel and bladder dysfunction, consistent with transverse myelitis, and was OB-negative. Her

Table 1 Comparison of AQP4 antibody-positive and AQP4 antibody-negative Thai patients with idiopathic inflammatory demyelinating CNS diseases^a

Variables/diagnosis	AQP4 antibody-positive (n = 53)	AQP4 antibody-negative (n = 82)	p Value
Sex, F/M (% F)	50/3 (94.3)	55/27 (67.1)	<0.0001
Demographic data, mean ± SD			
Age at onset, y	37.0 ± 15.5	35.9 ± 13.7	0.668
Disease duration, y	6.3 ± 4.9	5.1 ± 5.2	0.171
No. of attacks	6.4 ± 6.0	3.4 ± 3.1	0.001
Annual relapse rate, n/y	1.4 ± 1.2	1.6 ± 2.0	0.758
EDSS score	4.2 ± 2.5	3.2 ± 2.2	0.014
First clinical manifestation, n (%)			
Optic neuritis	21/53 (39.6)	35/82 (42.7)	0.725
Cerebrum dysfunction	1/53 (1.9)	2/82 (2.4)	0.832
Cerebellar dysfunction	1/53 (1.9)	0 (0)	0.212
Brainstem dysfunction	8/53 (15.1)	19/82 (23.2)	0.252
Myelitis	26/51 (51.1)	34/82 (41.5)	0.196
Brain MRI, n (% of those examined)			
Initial MRI brain abnormality	26/48 (54.2)	46/754 (61.3)	0.380
Initial MRI brain not meeting McDonald 2005 criteria	11/26 (42.3)	19/46 (41.3)	0.858
Spinal cord MRI			
Initial cord MRI, n (% of those examined)	25/42 (59.5)	29/55 (52.7)	0.504
Cord lesion >3 VBs, n (% of those examined)	30/42 (71.4)	20/55 (36.4)	<0.0001
No. of lesions, mean ± SD	1.2 ± 0.8	0.9 ± 0.7	0.0028
Length of lesion, VBs, mean ± SD	5.5 ± 4.2	2.8 ± 3.9	0.002
CSF analysis			
CSF OB, n (% of those with positive test results)	9/45 (20)	22/71 (31)	0.155
CSF neutrophil >5 cells/mm ³ , n (% of patients)	5/45 (11.1)	6/71 (8.5)	0.368

Abbreviations: AQP4 = aquaporin-4; CMS = classic multiple sclerosis; EDSS = Expanded Disability Status Scale; OB = oligoclonal immunoglobulin G bands; VB = vertebral body.

^a One patient with inadequate data and 5 patients with other diseases were excluded.

spinal cord MRI was performed later than 30 days after the onset. Subsequent brain MRI showed a midbrain lesion. Patient 9 had spinal cord syndrome, optic neuritis, and brainstem dysfunction. Brain MRI was suggestive of IIDCD. Her spinal cord MRI was inaccessible. Subsequent brain MRI showed periaqueductal involvement. Patient 10 had initial symptoms of optic neuritis with subsequent spinal cord dysfunction. Brain MRI was suggestive of IIDCD. She had never had spinal cord MRI. Patient 11 was diagnosed with probable primary progressive MS with a history of gradually progressive paraparesis over the 1-year period and brain MRI suggestive of IIDCD and was OB-negative. His spinal cord MRI showed only one short lesion (1 VB).

Taken together, these results show that all patients except patient 5 had certain clinical or MRI features that are relatively common in or unique to NMO, such as bilateral optic neuritis, recurrent optic neuritis with poor recovery, intractable hiccup/vomiting, periaqueductal lesions, or transverse myelitis. However, except for these features of NMO, there were no differences between patients with AQP4 antibody-negative and antibody-positive CMS (table 3).

Clinical characteristics of patients who were diagnosed with NMO but were AQP4 antibody-negative (AQP4 antibody-negative NMO). Five patients met all 4 Wingerchuk 2006 criteria and were diagnosed with NMO, but they were AQP4 antibody-negative. One of them presented with unilateral optic neuritis followed by subsequent attacks of optic neuritis, myelitis, or brainstem lesions. Brain MRI at onset was normal, but spinal cord MRI showed entire cord involvement. The patient was OB-positive and positive for ANA (titer 1:640). Two other patients had recurrent attacks of optic neuritis and transverse myelitis (>3 VBs) with normal brain MRI results at onset. Both were OB-negative. The remaining 2 patients had optic neuritis, brainstem lesions, and myelitis. Brain MRI showed a few white matter spotty lesions and did not meet Paty criteria, but spinal cord MRI revealed long cord lesions (>3 VBs). Both were OB-negative.

DISCUSSION The AQP4 antibody seroprevalence in our Thai patients with IIDCDs was 39.3%. This figure is remarkably higher than those in Western countries where cases of NMO constitute less than 10% of total IIDCDs and is still higher than available Asian data, such as those in Japanese cohorts¹⁵ and the 33.1% in 236 consecutive Korean patients with IIDCD seen at a major referral center.²⁶ The very high prevalence of the NMO-specific autoantibody in the present study is probably due to the low prevalence of MS in this region.¹⁶ In addition, because MS is a much milder disease, access issues might disproportionately inflate the proportion of cases of NMO in this non-population-based study. However, the high proportion of AQP4 antibody-positive patients in this Thai study has important clinical implications.

AQP4 is a dominant water channel in the CNS and is abundantly expressed on astrocytic endfeet.²⁷ Neuropathologic and CSF analyses in NMO demonstrated massive destruction of astrocytes in acute NMO lesions, but there were no such changes in MS.^{28,29} More recent experimental studies clearly showed that AQP4 antibody derived from patients with NMO, predominantly of the IgG1 subclass, ac-

Table 2 Clinical and laboratory data of patients who were diagnosed with CMS but were AQP4 antibody-positive

Patient	Sex/age at onset, y	Initial symptoms	All location of attacks	OB	Brain MRI compatible with Paty/Barkhof criteria	Spinal MRI findings
1	F/16	Unilateral ON	Bilateral ON, cord, brainstem	Negative	Yes/yes	2 VBs in length
2	F/18.1	Intractable hiccup/vomiting	Recurrent brainstem	Positive	Yes/yes	Never done because of no cord symptoms
3 ^a	F/16.9	Unilateral ON	Bilateral ON, brainstem, cord	Negative	Yes/yes; follow-up brain MRI showed fourth ventricular + brainstem lesions (at attack after the time of the present study)	Inaccessible
4 ^a	F/21.4	Bilateral ON	Bilateral ON, cord	Negative	Yes/yes	Inaccessible; a long cord lesion (>3 VBs) (at attack after the time of the present study)
5	F/32.6	Cerebellar dysfunction	Cerebellar, brainstem	Positive	No/no	Never done because of no cord symptom; no NMO feature by the time of the present study
6 ^a	F/26.6	Cerebral dysfunction	ON, cerebrum	Positive	Yes/no; follow-up brain MRI showed a periaqueductal lesion	Not done at relapse
7	F/22.5	Unilateral ON	Unilateral ON, brainstem	Negative	Yes/no	Not done at relapse
8 ^a	F/24.7	Unilateral ON	Bilateral ON, cord, brainstem	Negative	Yes/yes; follow-up brain MRI showed a periaqueductal, midbrain lesion	Not done at relapse
9 ^a	F/30.8	Spinal cord syndrome	Bilateral ON, cord, brainstem	Positive	Yes/no; follow-up brain MRI showed a periaqueductal lesion	Inaccessible
10	F/36.3	ON + subsequent TM	Bilateral ON, cord, brainstem	Negative	Yes/yes	Inaccessible
11 ^a	M/43.3	Progressive paraparesis	Cord	Negative	Yes/yes; follow-up brain MRI showed a periaqueductal lesion	1 VB in length

Abbreviations: AQP4 = aquaporin-4; CMS = classic multiple sclerosis; NMO = neuromyelitis optica; OB = oligoclonal immunoglobulin bands; ON = optic neuritis; TM = transverse myelitis; VB = vertebral body.

^a Patients who had additional MRI findings in figure 2.

tivated the complement pathway efficiently and caused astrocytic necrosis in cell culture and NMO-like lesions in animals with experimental autoimmune encephalomyelitis.³⁰ Purified IgG from AQP4 antibody-negative patients did not induce those morphologic changes. These findings clearly indicate that AQP4 antibody is pathogenic and NMO de-

fined by AQP4 antibody is a clinical entity distinct from MS.

The clinical and laboratory features of our AQP4 antibody-positive patients were similar to those reported previously: high female/male ratio (16:1), longitudinally extensive transverse myelitis with high Expanded Disability Status Scale scores and frequent

Figure 2 MRI findings

VB = vertebral body.

Table 3 Comparison of patients with AQP4 antibody-positive and AQP4 antibody-negative CMS

Variables/diagnosis	AQP4 antibody-negative (n = 35)	AQP4 antibody-positive (n = 11)	p Value
Sex, F/M (% F)	25/10 (71.4)	10/1 (90.9)	0.186
Demographic data, mean $\pm$ SD			
Age at onset, y	32.8 $\pm$ 14.7	26.3 $\pm$ 8.7	0.082
Disease duration, y	7.0 $\pm$ 6.1	8.3 $\pm$ 5.0	0.507
No. of attacks	4.83 $\pm$ 3.17	6.7 $\pm$ 5.4	0.287
Annual relapse rate, n/y	1.3 $\pm$ 1.6	1.3 $\pm$ 1.4	0.927
EDSS score	3.4 $\pm$ 2.2	3.6 $\pm$ 2.5	0.727
First clinical manifestation, n (%)			
Optic neuritis	13/35 (37.1)	6/11 (54.5)	0.307
Cerebrum dysfunction	1/35 (2.9)	1/11 (9.1)	0.377
Cerebellar dysfunction	0 (0)	1/11 (9.1)	0.071
Brainstem dysfunction	12/35 (34.3)	1/11 (9.1)	0.105
Myelitis	11/35 (31.4)	3/11 (27.3)	0.794
Brain MRI, n (% of those examined)			
Initial MRI brain abnormality	21/33 (63.6)	5/11 (45.5)	0.288
Initial MRI brain not meeting McDonald 2005 criteria	13/34 (38.2)	4/11 (36.4)	0.911
Spinal cord MRI			
Initial cord MRI, n (% of those examined)	6/20 (30)	1/4 (25)	0.841
No. of lesions, mean $\pm$ SD	0.7 $\pm$ 0.7	0.8 $\pm$ 1.5	0.952
Length of lesion, VBs, mean $\pm$ SD	1.1 $\pm$ 1.0	0.5 $\pm$ 1.0	0.326
CSF analysis			
CSF OB, n (% of those with positive test results)	17/29 (58.6)	4/11 (36.4)	0.208
CSF neutrophil > 5 cells/mm ³ , n (% of patients)	2/29 (6.9)	2/11 (18.2)	0.692

Abbreviations: AQP4 = aquaporin-4; CMS = classic multiple sclerosis; EDSS = Expanded Disability Status Scale; OB = oligoclonal immunoglobulin bands; VB = vertebral body.

relapse (about 1.0/year on average), and CSF pleocytosis.³¹ Of the patients who fulfilled Wingerchuk 2006 criteria, nearly 80% were AQP4 antibody-positive. Meanwhile, in contrast with previous studies, age at onset and frequency of brain MRI abnormalities in those meeting McDonald Criteria and CSF OB were not different between AQP4 antibody-positive and AQP4 antibody-negative Thai patients with IIDCD. For 11 patients who we diagnosed with CMS but who were AQP4 antibody-positive, in retrospect, all but one had clues that they might have had NMO. In most of these, spinal cord MRI was not done or was inaccessible. Thus, we should incorporate brain and spinal cord MRI in the initial diagnostic workup to differentiate NMO from CMS. Moreover, regardless of clinical, MRI, or laboratory findings, we should maintain a high index of suspicion for NMO, espe-

cially in Asian countries, where NMO is a common cause of IIDCDs.

The AQP4 antibody is highly sensitive and specific for detecting NMO, but extensive analyses of AQP4 antibody-positive patients revealed that in addition to those with typical NMO, there are AQP4 antibody-positive patients with recurrent optic neuritis or longitudinally extensive myelitis alone or certain brain lesions.^{10–13,32,33} We classified those patients as ONMOSD in the present study. Our definition of ONMOSD in the present study, however, is different from that of NMOSD originally proposed by investigators at the Mayo Clinic in that both positive serology and clinical features are used to define this entity rather than clinical features only. It is likely that other investigators would have suspected that many of the patients diagnosed with CMS and CIS in the present study had ONMOSD and established this diagnosis by testing for AQP4 antibody. Some of our patients with ONMOSD may have pre-NMO, which first presented with optic neuritis or longitudinally extensive myelitis. Although none of them have fulfilled Wingerchuk 2006 criteria for definite NMO to date, we need longitudinal data for each patient to clarify the evolution of NMO.

In Asian countries, we have commonly termed cases of recurrent optic neuritis and myelitis OSMS.^{14,34} However, so-called OSMS comprises NMO and MS with opticospinal presentation.³⁵ Because therapy for NMO is different from that for MS,^{36–38} the differential diagnosis is important. In the present study, we defined OSMS as MS with selective opticospinal involvement (optic neuritis and acute myelitis with a spinal cord MRI lesion not extending over >3 VBs and with normal brain MRI) to distinguish it from NMO. With the application of the criteria for OSMS and other diseases in the sequence described in Methods, in the present study, in addition to NMO and ONMOSD, we detected AQP4 antibody-positive patients in all other groups (57% in OSMS, 24% in CMS, and 6% in CIS). At the time of this study, those patients did not meet the criteria for NMO or ONMOSD in the absence of AQP4 antibody serologic status mainly because they had brain lesions, some of which met the MRI findings in the McDonald 2005 criteria,^{21,39} and they never underwent MRI, had a delayed spinal cord MRI examination, or lacked long cord lesions. Alternatively, spinal cord lesions may be shorter and AQP4 antibody may be undetectable in patients receiving immunosuppressive drugs.⁴⁰ Moreover, some AQP4 antibody-positive patients do not have any known features of NMO or ONMOSD at the time of diagnosis such as patient 5 in table 2. We may

misdiagnose such patients with MS and probably treat them with MS disease-modifying drugs. Unlike acute exacerbations in MS, those in NMO are often refractory to high-dose corticosteroids and need plasma exchange to hasten recovery.³⁸ Interferon- β is not efficacious in preventing relapse in NMO.^{7,8} Conversely, we diagnosed 5 patients with AQP4 antibody-negative NMO. One of them had been treated with interferon- β over 10 months. Two other patients were treated with azathioprine, which might have lowered AQP4 antibody levels below the detection limit as mentioned previously.⁴⁰

In the present study, the male/female ratio for patients with AQP4 antibody-negative CMS was 2.5, which was lower than that in our previous report (6.2), whereas positive OB tests (58.6%) in these patients were more frequent than in patients in the previous study (21%)³¹ and in patients with AQP4 antibody-positive CMS in the present study. We did our previous study without AQP4 antibody status, and thus we might have misdiagnosed many patients with AQP4 antibody-positive NMO or ONMOSD as CMS. Our AQP4 antibody-negative, OB-positive patients with CMS were very similar to patients with MS in Western countries.

Our study revealed that no single criterion can perfectly distinguish NMO from MS, and we must consider all clinical, CSF, and MRI findings as well as AQP4 antibody status in determining the optimal treatment for a patient, which is quite different for NMO than for MS. However, AQP4 antibodies are highly specific and positive test results should weigh strongly in favor of a diagnosis of NMO.

AUTHOR CONTRIBUTIONS

Dr. Siritho: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data, statistical analysis. Dr. Nakashima: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, study supervision, and obtaining funding. Dr. Takahashi: analysis or interpretation of data, contribution of vital reagents/tools/patients, and acquisition of data. Dr. Fujihara: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, study supervision, and obtaining funding. Dr. Prayoonwiwat: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, contribution of vital reagents/tools/patients, acquisition of data, study supervision, and obtaining funding.

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