Clinical Features and Sera Anti-Aquaporin 4 Antibody Positivity in Patients with Demyelinating Disorders of the Central Nervous System from Tianjin, China

Chun-Sheng Yang,¹ Da-Qi Zhang,¹ Jing-Hua Wang,¹ Wei-Na Jin,¹ Min-Shu Li,¹ Jie Liu,¹ Cun-Jin Zhang,¹ Ting Li,¹ Fu-Dong Shi^{1,2} & Li Yang¹

¹ Department of Neurology and Tianjin Neurological Institute, Tianjin Medical University General Hospital, Tianjin, China

² Department of Neurology and Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ, USA

Keywords

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Correspondence

L. Yang, M.D., Ph.D., Department of Neurology and Tianjin Neurological Institute, Tianjin Medical University, Tianjin 300052, China.

Tel.: +86-22-608-14602;

Fax: +86-22-608-17471;

E-mail: yungli2001@yahoo.com

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SUMMARY

Aims: To investigate the clinical characteristics and sera anti-aquaporin 4 (AQP4) antibody positivity in patients with inflammatory demyelinating disorders (IDDs) of the central nervous system (CNS) in Tianjin, China. **Methods:** We retrospectively evaluated 234 patients with IDDs including neuromyelitis optica (NMO), recurrent optic neuritis (rON), longitudinally extensive transverse myelitis (LETM), clinically isolated syndrome (CIS), and multiple sclerosis (MS) groups. Sera from 217 patients were determined for AQP4-Ab. The clinical characteristics and sera anti-AQP4 positivity were compared. **Results:** The IDDs comprised 63 MS, 51 NMO, 56 LETM, 10 rON, and 54 CIS. Compared with MS, NMO had a higher frequency of occurrence in women, intractable hiccup and nausea (IHN), medullospinal lesion, longitudinally extensive spinal cord lesions (LESCL) and bilateral ON, disease onset at a later age, and worsening residual disability. AQP4-Ab-positive rates were 84.1% and 69% in NMO and NMO spectrum disorders (NMOSD), respectively, whereas it was undetectable in all of the MS sera samples. **Conclusions:** We comprehensively contrast the distinct clinical features of MS, NMO, and NMOSD in our center. A sensitive AQP4-Ab assay is necessary for the early diagnosis of NMOSD in our patients. Neither medullospinal lesion nor IHN is unique in NMO.

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Introduction

Inflammatory demyelinating disorders (IDDs) of the central nervous system (CNS) occur throughout the world. Multiple sclerosis (MS) and neuromyelitis optica (NMO) are the most common diseases among these disorders. Considering the different treatments of these two diseases, early diagnosis is very important to reduce the relapse and residual disability associated with the diseases [1]. Both the discovery of the serum immunoglobulin G antibody (NMO-IgG), which recognizes the astrocyte aquaporin 4 (AQP4) water channel, and the evidence that AQP4-IgG is involved in the development of NMO confirmed the belief that NMO is a separate disease from MS [2]. Some clinical and radiological features are also helpful to distinguish NMO from MS, such as intractable hiccup and nausea (IHN) and medullospinal lesions as evinced by MRI [3].

Compared with Caucasians, Asian populations are found to be more susceptible to NMO. The NMO/MS ratio was much higher in

Asians than in Caucasians [4]. About 20% of patients with IDDs were NMO in a study conducted in Japan [5]. However, there are very few reports delineating the disease phenotype found in mainland China. Similarly, there are few reports concerning the sera positivity of AQP4 antibody (AQP4-Ab) in the mainland China. In this study, we investigated the prevalence of sera AQP4-Ab and compared the clinical, serological, and radiological characteristics of patients in different disease groups among patients with CNS IDDs in Tianjin, the fourth largest city of China.

Materials and methods

Patients and Controls

From 2008 to 2012, there were 276 patients diagnosed with IDDs enrolled in our databank. 234 patients who have been continually monitored for over 1 year were recruited in this study. The patients were divided into several groups based on the criteria as

follows. The NMO spectrum disorders (NMOSD) group includes: (1) the NMO group ($n = 51$), in which patients met the revised diagnostic criteria proposed by Wingerchuk *et al.* in 2006 [6], (2) the longitudinally extensive transverse myelitis (LETM) group ($n = 56$) is characterized by myelitis with ≥ 3 vertebral segments (VB) termed longitudinally extensive spinal cord lesions (LESCL), with or without compatible brain lesions for NMO, based on the report of Pittock *et al.* in 2006 [7], these patients were divided into recurrent LETM (rLETM) and single-attack LETM (sLETM) groups, and (3) the recurrent optic neuritis (rON) group without brain lesions ($n = 10$). The MS group ($n = 63$) is defined by cases fulfilling all items of the McDonald Criteria of MS as revised in 2010 [8]. The clinically isolated syndrome (CIS) group ($n = 54$) is defined by cases with all single inflammatory demyelinating episodes except NMOSD.

For detection of the AQP4 antibody, the following sera samples were included: 217 sera samples from IDD patients in the relapsing stage, 20 sera samples from patients with miscellaneous neurological diseases, including cerebrovascular disease ($n = 4$), Guillain-Barré syndrome ($n = 4$), myasthenia gravis ($n = 4$), chronic inflammatory demyelinating polyradiculoneuropathy ($n = 4$), Parkinson's disease ($n = 2$), systemic lupus erythematosus ($n = 1$), and amyotrophic lateral sclerosis ($n = 1$). Twenty sera samples from healthy donors were obtained as additional controls. All patients gave consent to participate in the study.

Acquisition of Clinical Data

The information of personal accounts and clinical signs, visual acuities, Kurtzke Expanded Disability Status Scale (EDSS), blood and cerebrospinal fluid (CSF) laboratory data, visual evoked potentials (VEP) results, and MR images were recorded in the databank. The sera and CSF samples were collected following patient consent and kept in our sample bank at -80°C . The EDSS was performed by two neurologists, both certified by the Neurostatus for EDSS competency.

Poor visual outcome is defined as Snellen visual acuity ≤ 0.05 or visual field < 20 degrees upon the latest assessment by ophthalmologists. Blindness was defined as having no light perception. Poor neurological ability was defined as having an EDSS score ≥ 6.0 (not affected by recent relapse) or at least one eye blind upon the latest assessment. Brain MRI lesions were evaluated blindly and independently by two radiologists according to either the Barkhof criteria or Pittock report [7,9]. Brain and spinal MRI abnormalities

were defined as positive if they showed typical demyelinating lesions.

Detection of Antibodies against AQP4 and Other Autoantigens

AQP4-Ab was detected by the cell-based assay (CBA). A group of 293 human embryonic kidney (HEK) cells were transfected with either enhanced green fluorescent protein (EGFP)-tagged human AQP4-M23-cDNA or EGFP alone (the plasmids were donations from Professor Angela Vincent and Professor David Beeson, Nuffield Department of Clinical Neurosciences, University of Oxford), then incubated with the diluted serum. After washing, cells were fixed and then incubated with Alexa Fluor 568-conjugated anti-human IgG (Invitrogen-Molecular Probes, Paisley, UK). Cells were washed and imaged on the Olympus IX-71 fluorescence microscope. Binding of the sera to the cells was scored blindly and independently by two individuals on a scale of 0–4, based on the intensity of the stain and the colocalization of the green fluorescent AQP4 with that of the red fluorescent secondary anti-human IgG (Figure 1). Values of one and above were considered positive [10].

Antinuclear antibodies spectrum (ANAs), including antinuclear antibodies (ANA), anti-Sjögren's syndrome A (anti-SSA), anti-Sjögren's syndrome B (anti-SSB), and so on in the patient sera samples were tested by line immunoassay, LIA (HUMAN-IMTEC, Berlin, Germany).

Statistical Analysis

We applied Mann-Whitney *U* test for quantitative data and chi-squared test or Fisher exact test for qualitative data. A *P*-value of < 0.05 is considered statistically significant. We used logistical regression to evaluate whether the presence of medullosplinal lesions increased the odds of a patient with NMO or LETM having an episode with nausea or hiccups.

Results

Demographic and Clinical Features

The city of Tianjin is the fourth largest city in China with a registered population of 13 million. During the years 2008–2012, a total of 276 patients with IDDs were treated by our clinic. A total

Figure 1 Anti-AQP4 antibody binds to aquaporin 4 (AQP4). AQP4-expressing cells were incubated with serum detected, followed by goat antiserum specific for IgG. The colocalization of the surface-expressed AQP4 (green) and the anti-IgG antibody (red) indicates that the antibodies are against AQP4. (Original magnification $\times 200$).

of 234 cases (166 female patients and 68 male patients) were monitored for at least a year or longer.

The demographic and clinical features of all the cohort patients were summarized in Table 1. This patient cohort included only patients from the mainland China population who were all Han ethnicity. The female to male ratio for all patients was 2.4, and the NMO group ranked the first with 9.2. The frequency of NMOSD in the cohort was 50%, with a MS/NMOSD ratio of 0.54. The median age of onset in the present cohort patient was 42 years (range 15–79), and the median duration of disease was 3.6 years (range 1–23). Compared with MS, NMO and LETM had significantly higher EDSS scores at the latest patient follow-up visit.

Radiological and Laboratory Features

Majority of the patients had MRI of brain or spine, 76.1% and 83.3%, respectively. Brain MRI abnormalities were observed in 100% of patients with MS, which was the highest among all of the groups. All patients with NMO presented with LESCL, but none LESCL in the MS group. More than half of the patients in the study had lumbar puncture (53.8%) and VEP studies (58.5%). ANAs were detectable in 65.7% of NMO, which was higher than that of all the other groups.

Sera Anti-AQP4 Ab Positivity

Among the 234 patients, 217 patients gave consent to test the serum for AQP4 antibody. Binding of the AQP4 antibody in the sera to the cells was scored from 0 to 4, based on the intensity of the stain and the colocalization of the green fluorescent AQP4 with that of the red fluorescent secondary anti-human IgG (Figure 1). Values of 1 and above were considered positive. The AQP4-Ab was present in 31.8% of the 217 sera samples, as shown in the CBA (Table 2). When the seropositive rates in different groups were compared, the respective positive frequencies were as follows: NMO group 84.1%, rLETM group 76.9%, and LETM 40%. The MS, CIS, healthy control, or miscellaneous disease groups all yielded negative results (Table 2).

Table 2 Anti-aquaporin 4 antibody-seropositive rate for each patient group

Patient/control groups	Number of subjects	Number seropositive for anti-aquaporin 4 antibody (seropositivity rate,%)
NMO spectrum	100	69 (69%)
NMO	44	37 (84.1%)
Relapsing LETM	26	20 (76.9%)
Single attack of LETM	28	8 (40%)
Recurrent ON	10	4 (40%)
CIS	54	0 (0%)
Single attack of Brain	14	0 (0%)
Single attack of ATM	29	0 (0%)
Single attack of ON	11	0 (0%)
MS	63	0 (0%)
Healthy subjects	20	0 (0%)
Miscellaneous diseases	20	0 (0%)

NMO, neuromyelitis optica; LETM, longitudinally extensive transverse myelitis; ON, optic neuritis; CIS, clinically isolated syndrome; ATM, acute transverse myelitis; MS, multiple sclerosis.

Comparison between NMO and MS

When NMO and MS were compared, NMO had a higher ratio of occurrence in females to males than MS ($P = 0.001$), and a later disease onset age ($P = 0.001$). In addition to the specific AQP4-Ab, more ANA-positive samples were found in NMO compared with MS ($P = 0.001$). NMO exhibited an increasingly severe disease progression with a higher annual relapse frequency in the first 2 years following diagnosis ($P < 0.001$). EDSS was assessed at the disease onset ($P = 0.001$) or at the latest follow-up ($P < 0.001$). Furthermore, more patients with NMO suffered bilateral and bilateral simultaneous ON ($P < 0.001$). NMO showed a comparatively poor neurological outcome ($P < 0.001$) and a higher frequency of IHN ($P = 0.044$) and medullospinal lesion on MRI ($P = 0.013$) (Table 3).

Table 1 Clinical features of patients with idiopathic inflammatory demyelinating disorders in Tianjin, China

	MS (63)	NMO (51)	LETM (56)	rON (10)	CIS (54)
Gender: F/M	40/23 (1.7)	46/5 (9.2)*	42/14 (3)	7/3 (2.3)	31/23 (1.4)
Age at onset, median (range), years	35 (15–63)	43 (20–74)*	50 (17–79)**	46 (15–65)*	42 (16–77)*
Disease duration, median (range), years	5.6 (1–20.5)	5.7 (1–20)	4.6 (1.5–30)	3.9 (2.3–23)	2.3 (1–10)
Annual relapse rate, median (range)	0.4 (0.05–1.6)	0.42 (0.05–2.0)	0.4 (0.1–2.6)	0.3 (0.1–1.3)	NA
EDSS score, median (range)	2 (0–7)	4 (1–10)**	4 (0–10)**	3 (2–4)	2 (0–6)
CSF marked pleocytosis (>50 cells/ μ l) (%)	1/34 (2.9)	2/20 (10)	5/36 (13.9)	0/2 (0)	1/34 (2.9)
OCBs (%)	10/19 (52.6)	2/7 (28.6)	5/13 (38.5)	0/0 (0)	0/18 (0)*
VEP (%)	26/35 (74.3)	21/24 (87.5)	4/28 (14.3)*	10/10 (100)	22/40 (55)
Brain MRI abnormalities (%)	63/63 (100)	18/51 (35.3)**	5/35 (14.3)**	0/10 (0)**	24/36 (66.7)*
Spinal MRI abnormalities (%)	44/49 (89.8)	51/51 (100)*	56/56 (100)*	0/3 (0)*	30/36 (83.3)
LESCL (%)	0/49 (0)	51/51 (100)**	56/56 (100)**	0/3 (0)	0/3 (0)
ANAs (%)	15/51 (29.4)	23/35 (65.7)*	15/46 (32.6)	2/6 (33.3)	11/50 (22)

MS, multiple sclerosis; NMO, neuromyelitis optica; LETM, longitudinally extensive transverse myelitis; rON, recurrent optic neuritis; CIS, clinically isolated syndrome; EDSS, Kurtzke Expanded Disability Status Scale; CSF, cerebrospinal fluid; OCBs, oligoclonal bands; VEP, visual evoked potential; MRI, magnetic resonance imaging; LESCL, longitudinally extensive spinal cord lesions; ANAs, antinuclear antibodies spectrum. Value was significant in comparison with MS. * $P < 0.05$, ** $P < 0.001$.

Comparison between Groups Divided by Sera Anti-AQP4 and/or ANAs Antibodies Positivity

When the AQP4-Ab-seropositive NMO and AQP4-Ab-seronegative NMO were compared, no significant differences were found between the two groups (Table 4). When NMO combined with LETM (either sLETM or rLETM) in one group, AQP4-Ab-positive and AQP4-Ab-negative cases were compared, showing that the increased female predominance was slightly higher in AQP4-Ab-positive patients than in AQP4-Ab-negative patients ($P = 0.025$). No other significant differences were found, even though the AQP4-Ab-positive patients in this group represented a slightly more severe neurological state without statistic difference ($P = 0.058$) (Table 5). There was no significant correlation between the AQP4-Ab scores and the annual relapse rates or EDSS in the patients with NMO or LETM. Also, no correlation was found between the lesion length of spinal cord on MRI and the AQP4-Ab values.

When the analysis focused on the ANAs-positive (23/35) or ANAs-negative (12/35) NMO, strong differentiation was found between the two groups in terms of female predominance and percentage of poor visual ability ($P < 0.001$) (Table 6).

There were 43 patients with NMO or LETM who tested for both AQP4-Ab and ANAs, and, of them, 30 were positive for both antibodies and 13 were negative for both antibodies. Analysis showed more patients with poor neurological outcome and relapsing course having higher EDSS scores and more women in both antibody-positive groups compared with those antibody-negative group for both antibodies (Table 7).

Association of IHN and Medullospinal Lesion on MRI

In the present cohort, there were a total of 16 patients suffering from IHN, including 8 patients with NMO, 5 patients with LETM, 2 patients with MS and 1 patients with CIS. Almost all these patients exhibited medullospinal lesions on MRI except one NMO patient with lesion only on the cervical spine. NMO had significantly higher frequency of IHN than that of MS ($P = 0.044$).

When focusing on the medullospinal lesion on MRI, there were 12/51 NMO and 12/56 LETM patients with medullospinal lesion as evinced by MRI, which was more than that found in patients with MS (2/63) and CIS (4/54) (Figure 2A, B). The sagittal and axial views of MRI revealed that the linear medullary lesions involved the central canal or pericanal regions in NMO and LETM, and the lesions rostrally extended into the medullary floor of the fourth ventricle and area postrema (AP) (Figure 2B, D). All the medullospinal lesions in patients with NMO and LETM extended to the cervical cord, and involved more than three VB. On the contrary, none of the medullospinal lesions in the CIS and MS groups extended to more than three VB or involved the medullary floor of the fourth ventricle or AP. (Figure 2A, C). Based on a logistic regression model with medullospinal lesion as the predictor and IHN as the event, the odds of IHN symptoms being present were an estimated 112 times greater with presence vs. absence of medullospinal lesions (95% CI 5.31–2389; $P = 0.002$).

Table 3 Clinical, serological, and radiological characteristics of the patients with NMO and MS

	NMO (51)	MS (63)	<i>P</i>
Gender: M/F	5/46	23/40	0.001
Age at onset, median (range), years	43 (20–74)	35 (15–63)	0.001
Annual relapse rate, median (range)	0.42 (0.05–2.0)	0.4 (0.05–1.6)	0.121
Annual relapse rate in the first 2 years, median (range)	0.5 (0–3)	0.5 (0–1.5)	0.001
Bilateral optic neuritis, (% of those existed)	38/49 (77.6)	6/20 (30)	0.001
Bilateral simultaneous optic neuritis, (% of those existed)	25/46 (54.3)	1/20 (5)	0.001
No. of patients with poor visual outcome, (% of those existed)	23/46 (50)	5/20 (25)	0.103
ANAs positive/tested (%)	23/35 (65.7)	15/51 (29.4)	0.001
CSF marked pleocytosis (>50 cells/ μ l) (%)	2/20 (10)	1/34 (2.9)	0.548
OCBs positive/tested (%)	2/7 (28.6)	10/19 (52.6)	0.391
VEP abnormal (%)	21/24 (87.5)	26/35 (74.2)	0.326
Brain MRI abnormalities (%)	18/51 (35.3)	63/63 (100)	<0.001
Spinal MRI abnormalities (%)	51/51 (100)	44/49 (89.8)	0.025
Length of T2W hyperintense abnormalities on MRI spine (VB, median/range)	5 (3–14)	2 (1–2.5)	0.001
LESCL (%)	51/51 (100)	0/49 (0)	<0.001
EDSS score at onset, median (range)	4.5 (1–10)	3 (0–9)	0.001
EDSS score at latest follow-up, median (range)	4 (1–10)	2 (0–7)	<0.001
Cases of death, attack related (%)	2/51 (3.9)	0/63 (0)	0.198
Poor neurological outcome (%)	31/51 (60.8)	10/63 (15.9)	0.001
IHN (%)	8/51 (15.7)	2/63 (3.2)	0.044
Medullospinal lesion on MRI (%)	12/51 (23.5)	4/63 (6.3)	0.013

NMO, neuromyelitis optica; MS, multiple sclerosis; ANAs, anti-nuclear antibodies spectrum; CSF, cerebrospinal fluid; OCBs, oligoclonal bands; VEP, visual evoked potential; MRI, magnetic resonance imaging; VB, vertebral segments; LESCL, longitudinally extensive spinal cord lesions; EDSS, Kurtzke Expanded Disability Status Scale; IHN, intractable hiccup and nausea.

Table 4 Clinical, serological, and radiological characteristics of patients with NMO divided into AQP4-Ab-seropositive and AQP4-Ab-seronegative groups

	Aquaporin-4 antibody-positive NMO (37)	Aquaporin-4 antibody-negative NMO (7)	<i>P</i>
Gender: M/F	3/34	1/6	0.513
Age at onset, median (range), years	46 (20–74)	34 (20–53)	0.058
Annual relapse rate, median (range)	0.46 (0.05–2)	0.37 (0.25–1.0)	0.685
Annual relapse rate in the first 2 years, median (range)	0.5 (0–3)	0.5 (0–1.5)	0.454
ON at onset (%)	24/37 (64.9)	2/7 (28.6)	0.103
ATM at onset (%)	11/37 (29.7)	3/7 (42.9)	0.662
Brain at onset (%)	0/37 (0)	1/7 (14.3)	0.159
Simultaneous ON and ATM at onset (%)	2/37 (5.4)	1/7 (14.3)	0.413
Bilateral optic neuritis (%)	30/36 (83.3)	5/7 (71.4)	0.597
Bilateral simultaneous optic neuritis (%)	20/36 (55.6)	2/7 (28.6)	0.240
No. of patients with blindness in at least one eye (%)	17/25 (68)	2/7 (28.6)	0.091
Relapse ratio ON/ATM, median (range)	1.0 (0.2–6.0)	0.67 (0.17–1.0)	0.155
Length of T2W hyperintense abnormalities on MRI spine (VB, median/range)	5 (3–14)	5 (4–14)	0.210
ANAs positive/tested (% of those tested)	19/29 (65.5)	4/6 (66.7)	1.000
Cases of death, attack related (%)	2/37 (5.4)	0/7 (0)	1.000
EDSS score at onset, median (range)	4.5 (2–10)	4 (2–8.5)	0.483
EDSS score at latest follow-up, median (range)	3.5 (1–10)	4.5 (2–8.0)	0.868
Poor neurological outcome (%)	24/37 (64.9)	4/7 (57.1)	0.692
IHN (%)	5/37 (13.5)	2/7 (28.6)	0.893
Medullospinal lesion (%)	9/37 (24.3)	2/7 (28.6)	1.000

NMO, neuromyelitis optica; ON, optic neuritis; ATM, acute transverse myelitis; MRI, magnetic resonance imaging; VB, vertebral segments; ANAs, anti-nuclear antibodies spectrum; EDSS, Kurtzke Expanded Disability Status Scale; IHN, intractable hiccup and nausea.

Table 5 Clinical, serological, and radiological characteristics of patients with NMO and LETM divided into AQP4-Ab-seropositive and AQP4-Ab-seronegative groups

	Aquaporin-4 antibody positive (NMO+LETM <i>n</i> = 65)	Aquaporin-4 antibody negative (NMO+LETM <i>n</i> = 25)	<i>P</i>
Gender: M/F	7/58	8/17	0.025
Age at onset, median (range), years	46.5 (17–79)	45 (17–73)	0.333
Annual relapse rate, median (range)	0.46 (0.05–2.6)	0.47 (0.18–1.16)	0.517
Annual relapse rate in the first 2 years, median (range)	0.5 (0–3)	0.5 (0–1.5)	0.652
ANAs positive/tested (%)	30/53 (56.6)	7/22 (31.8)	0.075
Length of T2W hyperintense abnormalities on MRI spine (VB, median/range)	5 (3–22)	6 (3–17)	0.263
EDSS score at onset, median (range)	5 (1.5–10)	4.5 (1–8.5)	0.382
EDSS score at latest follow-up, median (range)	4 (1–10)	3 (0–10)	0.301
Poor neurological outcome (%)	34/65 (52.3)	7/25 (28)	0.058
IHN (%)	8/65 (12.3)	4/25 (16)	1.000
Medullospinal lesion (%)	18/65 (27.7)	4/25 (16)	0.228

NMO, neuromyelitis optica; LETM, longitudinally extensive transverse myelitis; ANAs, anti-nuclear antibodies spectrum; MRI, magnetic resonance imaging; VB, vertebral segments; EDSS, Kurtzke Expanded Disability Status Scale; IHN, intractable hiccup and nausea.

Discussion

To the best of our knowledge, this is the first study comprehensively documenting the clinical and immunological features of MS, NMO, and NMOSD in a large Neurological Center in mainland China. In our cohort, there was a relatively higher preva-

lence of NMOSD, which was remarkably higher than those found in Western studies [11,12]. The percentage of NMOSD patients reached 50%, which was found to be similar to the results from studies conducted in Japan [5], Thailand [13], and Taiwan [14]. The MS/NMO ratio in the cohort was 1.2, which was lower than those of Western reports [11,12]. This may be due to a low

Table 6 Clinical, serological, and radiological characteristics of patients with NMO divided into ANAs-seropositive and ANAs-seronegative groups

	ANAs positive NMO (23)	ANAs negative NMO (12)	<i>P</i>
Gender: M/F	0/23	4/8	0.002
Age at onset, median (range), years	49 (20–74)	40 (26–64)	0.251
Annual relapse rate, median (range)	0.36 (0.05–2.0)	0.74 (0.21–1.0)	0.269
Annual relapse rate in the first 2 years, median (range)	0.5 (0–3)	0.75 (0.5–1.5)	0.138
Aquaporin-4 antibody positive/tested (%)	19/23 (82.6)	10/12 (83.3)	1.000
Bilateral optic neuritis (% of those existed)	20/22 (90.9)	9/12 (75)	0.319
Bilateral simultaneous optic neuritis (% of those existed)	14/22 (63.6)	5/12 (41.6)	0.288
No. of patients with poor visual outcome (% of those recorded)	14/16 (87.5)	4/12 (33.3)	0.005
Length of T2W hyperintense abnormalities on MRI spine (VB, median/range)	5 (3–14)	5 (3–14)	0.887
EDSS score at onset, median (range)	4 (2–10)	4.5 (3–10)	0.487
EDSS score at latest follow-up, median (range)	4.5 (1–10)	4 (1.5–10)	0.843
Cases of death, attack related (%)	1/23 (4.3)	1/12 (8.3)	1.000
Poor neurological outcome (%)	17/23 (73.9)	6/12 (50)	0.261

ANAs, anti-nuclear antibodies spectrum; NMO, neuromyelitis optica; MRI, magnetic resonance imaging; VB, vertebral segments; EDSS, Kurtzke Expanded Disability Status Scale.

Table 7 Clinical, serological, and radiological characteristics of patients with NMO and LETM divided into both AQP4-Ab and ANAs-seropositive and ANAs-seronegative groups

	Both kinds of autoantibodies positive NMO+LETM(30)	Both kinds of autoantibodies negative NMO+LETM(13)	<i>P</i>
Gender: M/F	1/29	7/6	<0.001
Age at onset, median (range), years	49.5 (17–79)	45 (17–73)	0.384
Annual relapse rate, median (range)	0.37 (0.05–2.0)	0.75 (0.36–1.16)	0.088
Annual relapse rate in the first 2 years, median (range)	0.5 (0–3)	1.0 (0–1.5)	0.434
Relapsing cases n (%)	28/30 (93.3)	5/13 (38.4)	<0.001
Length of T2W hyperintense abnormalities on MRI spine (VB, median/range)	5 (3–14)	5 (3–17)	0.270
EDSS score at onset, median (range)	5.5 (2–10)	6 (1–8.5)	0.511
EDSS score at latest follow-up, median (range)	4.5 (1–10)	2 (0–8)	0.044
IHN (%)	3/30 (33.3)	3/13 (23.1)	0.511
Medullospinal lesion (%)	8/30 (26.7)	1/13 (7.7)	0.237
Poor neurological outcome (%)	10/27 (70.4)	2/12 (16.7)	0.004

NMO, neuromyelitis optica; LETM, longitudinally extensive transverse myelitis; MRI, magnetic resonance imaging; VB, vertebral segments; EDSS, Kurtzke Expanded Disability Status Scale; IHN, intractable hiccup and nausea.

prevalence of MS in this area [15,16]. Clinically, MS symptoms were relatively milder than those of NMO, which may disproportionately inflate the proportion of cases of NMO in this cohort for our study, which were population-based. The IDD had a high occurrence in women (female/male ratio, 2.4), and the female predominance was strong in NMO with a female to male ratio of 9.2, which was significantly higher than that of Western reports [11,12,17,18] and Asian studies [5,14,19]. The median age of onset in the present patient cohort was 42 years (range 15–79), which was also older than patients included in the previous reports [5,14,17–19].

Compared with MS, patients with NMO have a later onset age, female predominance, higher percentage of both bilateral ON and bilateral simultaneous neuritis, and longer length of spinal lesion on MRI in the present cohort. These findings were consistent with previous reports [20,21]. These characteristics may account for

worsening neurological outcome and higher EDSS scores of NMO either at disease onset or at the latest follow-up as compared with that of MS.

The AQP4-Ab seroprevalence in this cohort study was 31.8%, which is remarkably higher than those in Western-based country [11,12,22] reports and is similar to those of Asian-based country [14,23–25] studies. Sera AQP4-Ab-positive rate were 69% in NMOSD, whereas it was undetectable in all of the MS sera studied. Importantly, we demonstrate that AQP4-Ab is exclusively detected in NMOSD, reinforcing the notion that AQP4-Ab is closely associated with NMOSD and thus is helpful in distinguishing it from MS. However, we cannot exclude the possibility that sera samples are not large enough or CBA sensitivity is not high enough to reveal anti-AQP4-Ab positivity, which has been reported in other cohorts [13].

Figure 2 MRI features in patients with intractable hiccup and nausea (IHN). (A and C) The T2-hyperintense lesion (arrow) was limited in the medullospinal area and spared the medullary floor of the fourth ventricle or area postrema (AP) in the MS patient with IHN. (B and D) The T2-hyperintense lesion (arrows) in the upper cervical cord rostrally extended to the fourth ventricle and AP in the patient with NMO.

Within NMOSD, the AQP4-Ab was detectable in 37 of 44 (84.1%) NMO patients consistent with the previous reports ranging from 78–91% [10,13,24], which were higher than the results tested by tissue-based indirect immunofluorescence assay (IIFA) 19–73% [26]. This may be due to the difference of the amino acid sequences in the extracellular domains of AQP4 between the human and mouse AQP4. The sera AQP4-Ab-positive rate of 60.7% in the LETM group was found to be similar to the data from studies conducted in Taiwan [27] and Hongkong [23], but higher than those of Western studies ranging from 33.3% to 55% [10,11,20,28], and still remarkably higher than that of Korean reports [29]. The AQP4-Ab-seropositive frequency in rLETM is 76.9%, which is similar to that of Chan's report [23], but higher than 52% as found in Lennon's study [20]. These findings suggest Chinese patients with LETM are more likely to relapse or progress to NMO than those of other countries. In the study, a total of 29 patients suffered from acute partial transverse myelitis (APTM) in CIS. Compared with the high AQP4-Ab prevalence in LETM, no patients with APTM was found to be AQP4-Ab positive. Scott *et al.* [30] and Chan [26] shared similar results. All of these indicate that AQP4-Ab seems to differentiate the patients with LETM from those with APTM, thus having predictive and prognostic significance, suggesting that the length of the lesion in transverse myelitis is highly significant.

The ANAs-positive rate in the NMO group was significantly higher than that of MS. NMO patients with ANAs had a higher percentage of poor visual ability. Furthermore, NMO and LETM patients with both AQP4-Ab and ANAs showed higher EDSS scores, and more patients exhibited both relapsing course and poor neurological outcome. All these findings suggest patients

with ANAs seem to undertake a more intense autoimmune response and that ANAs-induced tissue damage may promote AQP4-induced pathology. ANAs or other inflammatory mechanisms may contribute to vascular damage induced by vasculitis or the disruption of the blood–brain barrier (BBB), which makes the AQP4-Ab accessible to the CNS and triggers the AQP4-Ab-mediated inflammatory cascade [31,32]. These findings may raise a prevailing notion of whether NMO is a complication of a systemic autoimmune disease with predominant CNS manifestations. However, in the present cohort, only a few differences were obtained between patients with or without ANAs in general. Previous studies showed AQP4-Ab prevalence did not differ between NMO and NMO combined with systemic autoimmune diseases; AQP4-Ab was exclusively detected in patients with NMOSD and not detected otherwise. Together, these findings support the hypothesis that NMO and systematic connective tissue diseases can be coexisting conditions that are clinically expressed in patients with autoimmune susceptibility [33].

Some studies showed IHN and medullospinal lesion are unique symptoms of NMO [3]. The present study showed both of them could be found in patients with MS. But compared with those of MS, NMO exhibited LESCL in cervical segment extending rostrally into the fourth ventricle and AP. AP expresses high levels of AQP4 and is thus preferential targets for NMO lesions. It seems anti-AQP4 antibody plays an important role in the pathogenesis of IHN. In addition, AP has a thin ependymal cover and is penetrated by convoluted capillaries that lack tight endothelial junctions forming a permeable BBB. This anatomical site could thus serve as a portal for circulating lymphocytes and cytokines entry into CNS. These may explain IHN in MS and AQP4-Ab-negative NMO.

In conclusion, our study revealed that patients with NMO or NMOSD had defining clinical features, which suggest a differentiation of their disease state from that of MS. High prevalence of sera anti-AQP4 antibody was found in Chinese patients with NMO and LETM. A sensitive anti-AQP4 antibody assay is necessary for the early diagnosis of NMOSD. Neither medullospinal lesion nor IHN is unique in NMO.

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Conflict of Interest

The authors declare no conflict of interest.

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