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Diagnostic utility of NMO/AQP4-IgG in evaluating CNS inflammatory disease in Thai Patients

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Abstract

Epidemiological studies in Thailand have reported that inflammatory demyelinating diseases (IDDs) commonly affect the optic nerve and spinal cord. We investigated the diagnostic utility of aquaporin (AQP)-4-IgG testing in 31 consecutive patients evaluated for CNS IDDs in 3 academic Thai hospital neurology clinics between February 2008 and January 2009. Patients were classified into 3 clinical diagnostic groups: Neuromyelitis optica (NMO, n=10) multiple sclerosis (MS n=5) and unclassified IDD (n=16). All sera were tested blindly by cell binding (Euroimmun) assay (CBA). Sera were also tested by indirect immunofluorescence assay (IFA) and ELISA (RSR/Kronus). After initial screening by CBA, AQP4-IgG was detected in 6 NMO patients (60%); 3 of the 4 seronegative cases were receiving immunosuppressants. AQP4-IgG was detected in 13 unclassified IDD cases (81%), but in no MS cases. Cell binding assay and ELISA were more sensitive than IFA (p=0.0004). The 81% seropositivity rate in “unclassified” patients suggests that AQP4 autoimmunity accounts for a significant proportion of Thai CNS inflammatory demyelinating disease, especially those with optic neuritis or transverse myelitis, with or without abnormal brain MRI, in whom a specific diagnosis or clear-cut treatment approach is unclear.

Keywords

demyelinating disease; Devic’s syndrome; autoimmune diseases; neuromyelitis optica; aquaporin-4; autoantibodies; multiple sclerosis; assays; diagnosis; sensitivity

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Introduction

Aquaporin-4 autoantibodies (AQP4-IgG) are sensitive and specific for the neuromyelitis optica (NMO) spectrum of inflammatory demyelinating CNS disorders (NMOSD).[1–3] Recurrent episodes of transverse myelitis without optic neuritis, or vice versa, are common manifestations; encephalopathies occur less commonly. [2,4] Accurate diagnosis of NMOSDs is critical because optimal treatments differ from multiple sclerosis. [5,6,7]

A 1994 epidemiological study in Thailand reported that inflammatory demyelinating diseases commonly affect the optic nerve and spinal cord. [8] Recent serological reports suggest that NMOSDs may account for a significantly greater proportion of inflammatory demyelinating CNS diseases than in the west.[8–10] Introduction of AQP4-IgG testing to neurology clinics of Thailand is enhancing diagnostic accuracy,[9,10] and is anticipated to improve therapeutic outcomes. We assessed the diagnostic utility of AQP4-IgG testing in patients with inflammatory demyelinating CNS diseases evaluated in neurology clinics at 3 academic Thai hospitals.

Methods

The study was approved by participating Thai clinics and Mayo Clinic Institutional Review Boards; written informed consent was obtained from all participating patients. Serum was collected from 31 consecutive patients evaluated for CNS inflammatory demyelinating diseases between February 1, 2008 and January 31, 2009. The patients were classified as: Group 1 (10 patients) fulfilling Wingerchuk 2006 NMO diagnostic criteria [10] (excluding AQP4-IgG serostatus); Group 2 (5 patients) fulfilling McDonald 2005 revised MS diagnostic criteria. Group 3 (16 patients) “unclassified” (i.e., distinction between MS and NMOSD, and therefore optimal treatment plan deemed unclear), included: optic neuritis (ON) and short segment myelitis with normal brain imaging or recurrent short segment myelitis with normal brain imaging (n=4); ON and longitudinally-extensive transverse myelitis (LETM) with brain abnormalities fulfilling Barkhof criteria (n=2); single episode LETM (n=3); recurrent LETM (n=4); recurrent ON (n=3).

All sera were tested in blinded fashion in the Neuroimmunology Laboratory at Mayo Clinic for AQP4-IgG using an AQP4-transfected cell binding assay (CBA, Euroimmun), indirect immunofluorescence assay (IFA) and ELISA.

CBA

HEK-293 cells transfected with M1 and M23 in kit form (EUROIMMUN, Luebeck Germany) were used to detect AQP4-specific IgG. Sera (1:10) were incubated for 30 minutes at room temperature and washed once for 5 minutes with PBS/Tween. Cells were then incubated with fluorescein-labeled goat anti-human-IgG for 30 minutes at 22°C, washed again with PBS/Tween, then coverslipped. Specific IgG binding was confirmed by comparison of binding to non-transfected cells. [3]

IFA

Sera were tested using 4 μm cryosections prepared from a composite of unfixed adult mouse kidney, stomach, cerebellum/midbrain permeabilised with 1% CHAPS, fixed with 10% phosphate-buffered formalin and blocked with 10% normal goat serum in PBS. Sera was diluted 1:60 in PBS containing 1% bovine serum albumin (BSA), and preabsorbed with bovine liver powder. Bound IgG was detected using fluorescein (FITC)-conjugated goat IgG specific for human-IgG (Southern Biotechnology, Birmingham, AL). [1] Sections yielding the NMO-typical immunofluorescence pattern were scored positive. The NMO-IgG titer was

determined by the reciprocal of the final dilution scored positive in doubling serum dilutions.

ELISA

serum (50 μL) and AQP4-biotin (25 μL) were added sequentially to ELISA plates (KRONUS/RSR), coated with human recombinant AQP4. After shaking at room temperature for 2 hours (500 rpm), the wells were aspirated and washed 3 times with buffer, and streptavidin-peroxidase conjugate (100 μL) was added. After shaking another 20 min at room temperature and repeated washing, tetramethylbenzidine substrate was added (100 μL). The plate was held in the dark for 20 min, and100 μL of stop solution was added. Optical density was read at 450 nm by plate reader (TECAN Infinite 200). Four calibrators included in each assay. AQP4 autoantibody concentrations (U/mL) were estimated from the calibration curve. [3]

Results of all 3 assays were compared using Cochran's Q and McNemar's tests.

Results

Group 1(NMO; n=10): Eight were female (mean onset age 42 years). Five (50%) had brain imaging abnormalities incompatible with MS criteria. The 7 available CSF samples had pleocytosis (median 13 cells/μL) and elevated protein (median 74 mg/dL). Two had supernumerary oligoclonal bands. AQP4-IgG testing by CBA identified 6 positive cases (sensitivity 60%). Three of the 4 seronegative patients were receiving immunosuppressive medication at serum drawing.

Group 2 (MS; n=5): All were female (mean onset age 38 years). The 4 available CSF samples had mild pleocytosis (range 0–7 cells/μL), and mildly elevated protein (median 46 mg/dL). Two had supernumerary oligoclonal band. No patient tested positive for AQP4-IgG. No patient was receiving immunosuppressive medication.

Group 3 (Unclassified IDD; n=16): Thirteen of 16 patients' sera (81%) yielded positive results by CBA. Demographic data, clinical and laboratory data for this group are summarized in table 1.

Cochran's Q test was performed to compare the results of the 3 assays (IFA, ELISA and CBA) performed on patients from groups 1 and 3 (Table 2). Significant differences among them were identified (p=0.0004). To investigate this difference we performed McNemar's test for paired proportions and found that the IFA was statistically different from CBA (p=002) and ELISA (p=008) but ELISA and CBA were not statistically different (p=08).

Discussion

As reported in 2 recent studies, NMOSDs are more common than MS in patients presenting to Thai Neurology Clinics for evaluation of inflammatory demyelinating CNS diseases. [9,10] This study provides further evidence for the utility of introducing AQP4-IgG testing for Thai patients undergoing Neurology Clinic evaluation for inflammatory demyelinating CNS diseases. The overall 81% seropositivity rate in the group of "unclassified" patients strongly provides further evidence that AQP4 autoimmunity may account for a majority of inflammatory demyelinating CNS disease in Thailand. Testing should be strongly considered in Thai patients with optic neuritis or transverse myelitis with or without abnormal brain MRI in whom a specific diagnosis or clear-cut treatment approach is unclear.

The 75% seropositivity in patients with optic neuritis, short rather than long-segment myelitis and normal brain MRI may relate to timing of spinal MRI. Two seropositive patients had optic neuritis and LETM with brain MRI lesions fulfilling Barkhof MS criteria. This finding is consistent with the North American finding that 10% of NMO patients (usually at relapse) have MRI brain lesions fulfilling Barkhof MS criteria.[12] AQP4-IgG detection in this "unclassified" group allows NMOSD diagnosis, predicts relapse and justifies immunosuppressant therapies aimed at relapse prevention rather than interferon-β. [2,5–7] Patients 6 and 11 [table 1] were receiving IFN-β for presumed MS but were reclassified as NMOSD after serological testing.

Despite the fact that all patients with classical MS in this study were seronegative, one could argue that screening all cases of CNS IDD for AQP4-IgG may be appropriate especially given the apparent high prevalence of NMOSD relative to MS in Thailand as compared to western countries. The immunomodulating agents (Interferon-β) are expensive and are currently not supported by the government for the treatment of MS. Given this fact, detection of AQP4-IgG in a patient would confirm a diagnosis of NMOSD and direct therapy toward immunosuppressant medications (with prednisone and azathioprine) that are much more affordable. The lower sensitivity of IFA and the significantly increased detection of NMO-IgG observed in this study by use of ELISA and CBA confirms the findings from a recent multicenter study. [3]

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Table 1

Clinical characteristics of patients in group 3: Unclassified inflammatory demyelinating disorders. The final AQP4-IgG result was considered positive when one or more tests were positive. Patient 6 and 11 were reclassified as NMO rather than multiple sclerosis, and were withdrawn from IFN-beta.

Patient no.	Sex/Age at onset	ON episodes	Myelitis episodes(MRI longest lesion length)	Brain lesions (Y/N); fulfilled Barkhof criteria (Y/N)	AQP-4-IgG serostatus				Therapy pre-testing
					IFA (Negative<120)	ELISA* (Negative <5IU/ML)	CBA*	FINAL RESULT	
1	F/50	8	12 (<3 segs)	Yes; No	−	−	+	Positive	Azathioprine
2	F/47	0	2 (<3 segs)	Yes; No	−	6.4	+	Positive	Azathioprine
3	F/55	2	1(<3 segs)	Yes; No	−	−	−	Negative	IFN-β
4	F/37	3	1 (<3 segs)	Yes; No	3840	126.0	+	Positive	Azathioprine
5	F/51	10	7 (>3 segs)	Yes; Yes	−	−	+	Positive	Mycophenolate mofetil
6	F/48	1	7 (>3 segs)	Yes; Yes	3840	39.3	+	Positive	IFN-β (no benefit); azathioprine plus prednisolone (long term remission)
7	F/21	0	1 (>3 segs)	Yes; Yes	−	−	−	Negative	Azathioprine
8	F/30	0	1 (>3 segs)	No	240	109	+	Positive	Prednisolone
9	F/63	0	1 (>3 segs)	Yes; Yes	−	9.6	+	Positive	None
10	F/30	0	8 (>3 segs)	No	240	36.7	+	Positive	Mycophenolate mofetil
11	F/40	0	2 (>3 segs)	Yes; Yes	−	16.6	+	Positive	IFN-β
12	F/55	0	3 (>3 segs)	No	−	7.0	+	Positive	Azathioprine
13	F/25	0	3 (>3 segs)	Yes; Yes	−	15.1	+	Positive	Azathioprine plus prednisolone
14	F/34	2	0	No	−	−	−	Negative	IFN-β
15	F/28	3	0	No	3840	>160	+	Positive	None
16	F/56	3	0	No	−	9.7	+	Positive	Cyclophosphamide

*AQP4 ELISA kit from RSR/Kronus Ltd. and CBA slides from Euroimmun.

Table 2

Comparison of IF, ELISA and CBA NMO/AQP4-IgG detection rates [Number (%) of each group positive]

Group	IF	ELISA (>5IU/ml)	CBA
1: NMO (n=10)	4 (40%)	5 (50%)	6 (60%)
2:MS (n=5)	0	0	0
3:Unclassified (n=16)	5 (31%)	11 (69%)	13 (81%)