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Short communication

Quantitative analysis of aquaporin-4 antibody in longitudinally extensive transverse myelitis

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ABSTRACT

Aquaporin-4 (AQP-4) antibody-positive longitudinally extensive transverse myelitis (LETM) is referred to as a neuromyelitis optica (NMO) spectrum disorder. We conducted an exploratory investigation of correlations between AQP-4 antibody serum levels, as determined by a fluorescent immunoprecipitation assay, and clinical characteristics in LETM. Expanded Disability Status Scores (EDSS) scores and number of segments of spinal cord involved were positively correlated to AQP-4 antibody levels. However, serum AQP-4 antibody levels were not correlated with the time to next attack or the conversion time of LETM to NMO, although seropositive LETM patients demonstrated a high conversion rate to NMO (78.1%).

1. Introduction

Neuromyelitis optica (NMO) is an acute or subacute demyelinating illness, which predominantly affects the optic nerves and spinal cord. Longitudinally extensive transverse myelitis (LETM; i.e. myelitis, which extends over a continuous lesion at least 3 vertebral segments in length) is the hallmark of NMO (Tobin et al., 2014). Aquaporin-4 (AQP-4) antibody, or NMO-immunoglobulin G (IgG), is an important diagnostic biomarker of definite and limited forms of NMO, collectively referred to as NMO spectrum disorders (NMOSD; Lennon et al., 2004; Jarius and Wildemann, 2013). This classification now includes seropositive patients with attacks of only optic neuritis or LETM (Wingerchuk et al., 2007). AQP-4 IgG detection has become an essential tool in the diagnosis of patients at high risk for NMOSD (Waters et al., 2012). However, qualitative detection rather than quantitative determination of AQP-4 antibody has been most widely used in clinical research on NMOSD. The aim of the present study was to investigate clinical features associated with a range of different AQP-4 antibody levels.

2. Methods

2.1. Subjects

We tested 72 patients with first-ever LETM who were admitted to the Neuroimmunology Center of Tianjin Medical University General Hospital between January, 2008 and March, 2013. The LETM group was characterized by myelitis affecting ≥3 vertebral segments, termed longitudinally extensive spinal cord lesions (LESCL), with or without the brain lesions of NMO, based on the report of Pittock et al. (2006). At the last follow-up, 30 patients fulfilled the Wingerchuk diagnostic criteria for NMO (Wingerchuk et al., 2006); 28 patients were diagnosed as recurrent LETM, and the remaining 14 patients were still diagnosed as monophasic LETM. This study was approved by the Ethics Committee of our hospital and all patients gave their consent to participate in the study.

Detailed clinical information and first episode blood samples were available from our database and the sample bank of Central Nervous System Demyelinating Disease, respectively. For detection of the AQP-4 antibody, the patients' blood samples were collected during the acute phase before introducing immunosuppressive medicine (intravenous corticosteroids). The median latency between blood sampling and the onset of LETM was 4 days (range 1–7). Ten serum samples from healthy donors were obtained to comprise the healthy control group (HCs). Twenty serum samples from patients with neurological diseases other than NMOSD (Guillain–Barré syndrome, 6; cerebral vascular infarction, 7; and encephalitis, 7) were evaluated as the disease control

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group. EDSS scores were calculated twice — when the blood samples were collected and at the last follow-up. Spinal MRI scans were performed in the acute phase (1.5- or 3.0-Tesla) of the first episode. Spinal MRI abnormalities were defined as positive if they showed typical demyelinating lesions. The lengths of spinal cord lesions, the time to next attack and the conversion time of LETM to NMO were recorded.

2.2. Fluorescent immunoprecipitation assay (FIPA) for AQP-4 antibody testing

Human embryonic kidney (HEK293) cells were planted in 175 cm^2 flasks. When the adherent cells' confluence reached 50%, the cultured HEK293 cells were transfected with EGFP-tagged AQP4 M23 plasmid (donated by the Department of Clinical Neurology of Oxford University) using polyethyleneimine (PEI). After 48 h of incubation, the transfected cells were lysed with extraction buffer (10 mM Tris–HCl pH 7.5, 100 mM NaCl, 1 mM EDTA, 1% Triton X-100, with a protease inhibitor cocktail added immediately before use). The extracted EGFP-AQP-4 protein was acquired after centrifugation. The fluorescent units (FUs) were assayed and adjusted to 500–600 FU/200 μL extract. Twenty microliter serum was incubated with 200 μL extract at 4 °C overnight, after which the antibody IgG was precipitated by the addition of 75 μL Protein A Sepharose (previously blocked with ultra-low fetal calf serum). After thorough washing, the beads with 150 μL extraction buffer were removed to 96-well black microtiter plates and the FU values (Excitation 488 nm, Emission 507 nm) were obtained on a Microplate reader (SYNERGY 2, BioTek, U.S.A.; Waters and Vincent, 2008; Yang et al., 2011). Assays on positive samples above 400 FU were repeated by titrating the sera. In the FIPA, we established a cut-off value corresponding to the mean FU of the healthy control sera plus three standard deviations, such that the cut-off value for our AQP4-Ab detection was 7.8 FU. All samples were tested in the same run and duplicate samples were redetected by the same operator for evaluating intra-assay variation. Data analysis was performed on the average of the two results. Three samples (a negative patient sample, a low positive patient sample, and a high positive patient sample in a previous cell-based assay) were tested as above in 2 independent runs. The intrarun coefficients of variability (%CV) were 2.23 and 2.31.

2.3. Statistical analysis

We used Spearman's rank correlation to explore potential correlations between AQP-4 antibody serum levels and clinical parameters such as EDSS scores, number of segments of spinal cord involved, time to next relapse, and conversion time of LETM to NMO. Correction for multiple testing was not performed due to the exploratory nature of this study. Student's *t*-test was used for between-group comparisons of AQP-4 antibody serum levels (with 10 at the bottom of the logarithmic transformation). Categorical variables were compared using the chi-squared test. The threshold for statistical significance was set at $p < 0.05$, $\alpha = 0.05$. All statistical analyses were performed using GraphPad Prism Version 5.

3. Results

3.1. Demographic, clinical, radiological and laboratory findings in patients with LETM

Of the 72 patients who presented with idiopathic LETM, AQP-4 antibodies were detected in 49 patients such that the positivity for AQP-4 antibody was 68%. Twenty disease control patients were negative in the FIPA. Sensitivity and specificity were 68% (95% CI 62.5%–73.5%) and 100% (95% CI 83%–100%), respectively. Detailed characteristics of the 49 seropositive LETM patients are listed in Table 1. All 72 cases were followed up over a one-year period.

Table 1
Demographic and clinical characteristics of 49 seropositive LETM patients.

	AQP-4 antibody-positive LETMn = 49
Sex (male/female)	7/42
Age at onset (years), mean ± SD	47.10 ± 15.78
Disease duration (months), mean ± SD	52.08 ± 22.67
EDSS at sample collection time, mean ± SD	6.03 ± 2.12
EDSS at the last follow-up, mean ± SD	3.16 ± 1.44
Number of segments of spinal cord involved at the first episode, median (range)	6 (3,18)
Number of brain lesions involved at the first episode, median (range)	1 (0,10)
AQP-4 antibody serum levels at first LETM (FU), median (range)	56 (8,940)
Time to next attack (irrespective of optic nerve, brain or spinal cord) (months), median (range)	12 (1,84)
Conversion period of LETM to NMO (months), median (range)	21.5 (3,74)

AQP-4 — aquaporin-4; EDSS — expanded disability status scale; FU — fluorescent unit; LETM — longitudinally extensive transverse myelitis; NMO — neuromyelitis optica.

3.2. AQP-4 antibody levels and clinical characteristics in seropositive LETM patients

The demographic and clinical features of the 49 AQP-4 antibody-positive patients can be summarized as follows. EDSS scores at the time of sample collection and at the last follow-up were both positively correlated with AQP-4 antibody levels (sample collection: $r = 0.463$, $p = 0.001$; last follow-up: $r = 0.424$, $p = 0.003$). The number of segments of spinal cord involved was weakly correlated with AQP-4 antibody levels ($r = 0.142$, $p = 0.039$). We made further comparisons of AQP-4 antibody levels according to the lesion length (10 segments) of spinal cord and found a significant difference ($p < 0.05$; Fig. 1). The time to next attack (irrespective of optic nerve, brain or spinal cord) was not correlated with AQP-4 antibody level ($r = -0.29$, $p = 0.073$; Fig. 2a, b, c, d).

3.3. Relationship of AQP-4 antibody status and conversion of LETM to NMO

At the last follow-up visit, 30 patients of the total cohort ($n = 72$) had progressed to NMO (25/32 seropositive; 5/14 seronegative, $p < 0.05$; Fig. 3). The median time to NMO conversion was 21.5 months (range 3–74). Nevertheless, the conversion time of LETM to NMO was not correlated with AQP-4 antibody level at the first episode ($r = -0.141$, $p = 0.458$). (Fig. 2e).

4. Discussion

In vitro studies have shown that NMO-IgG-mediated antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent

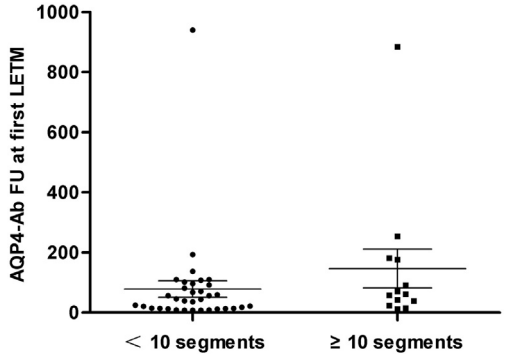

Fig. 1. Comparisons of AQP-4 antibody levels according to the lesion length (10 segments) of spinal cord. FU — fluorescent unit, LETM — longitudinally extensive transverse myelitis.