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Prevalence of anti-aquaporin 4 antibody in a diagnostic cohort of patients being investigated for possible neuromyelitis optica spectrum disorder in Western Australia

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ABSTRACT

Objective: To evaluate the prevalence of anti-AQP4 antibody in serum and CSF samples from patients being investigated for possible neuromyelitis optica spectrum disorder (NMOSD) referred to the PathWest State reference laboratory using a sensitive cell-based assay (CBA).

Background: NMOSD is an inflammatory CNS disease distinct from MS, which is relatively rare in Western countries. A proportion of patients with NMOSD have detectable serum IgG antibodies that target the water channel aquaporin-4 (AQP4-IgG), but the frequency varies in different populations studied and according to the assay method employed.

Methods: Sera or CSF from a diagnostic cohort of 196 consecutive patients with possible NMOSD which had previously been screened by indirect immunofluorescence (IIF) on primate cerebellum were re-tested for AQP4-IgG reactivity to the M1 and M23 isoforms of AQP4 using a commercial CBA. A control group of 205 patients with definite MS was also included in the study.

Results: Of the 196 patients, only 5 sera were AQP4-IgG positive, representing 2.6% of patients in the diagnostic cohort. All 5 AQP4-IgG positive patients fulfilled the 2015 revised diagnostic criteria for NMOSD and were females of varied ethnic origins, 4 of whom had longitudinally extensive transverse myelitis. The CBA confirmed AQP4-IgG positivity in the four patients previously reported as positive by IIF, and an additional patient with NMOSD who had previously been diagnosed as MS was also identified. None of the 205 MS sera were AQP4-IgG positive.

Conclusions: Our study confirms the utility and greater reliability of the M1/M23 CBA for detecting AQP4-IgG in patients with possible NMOSD, and indicates a prevalence of seropositive NMOSD in the Western Australian population similar to that in other Western populations.

1. Introduction

Aquaporin 4 (AQP4) is a water channel found in high density in astrocytic foot processes, particularly those in close proximity to the blood-brain barrier. AQP4 is believed to be the primary antigenic target in neuromyelitis optica (NMO), an antibody-mediated autoimmune disease of the central nervous system (CNS) (Lennon et al., 2004), mainly affecting the optic nerve and the spinal cord (Lucchinetti et al.,

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2002; Wingerchuk et al., 1999; Wingerchuk and Weinshenker, 2003). In the astrocytic plasma membrane, AQP4 exists as tetramers containing one or both of the 2 major isoforms: full length (M1-AQP4) and a shorter isoform (M23-AQP4). The AQP4–immunoglobulin G (AQP4-IgG) antibody is a well-characterized biomarker for neuromyelitis optica spectrum disorders (NMOSD) and has an important role in differentiating NMOSD from multiple sclerosis (MS) and for directing decisions for the use of treatment strategies (Tradtrantip et al., 2012). Early discrimination between NMO and MS is important, as it is now known that a number of disease-modifying agents used in MS may be harmful in NMO, whereas immunotherapy can be beneficial.

In 2015, the International Panel for NMO Diagnosis (IPND) unified the terms NMO and NMOSD, which was then further stratified into seropositive and seronegative NMOSD (Wingerchuk et al., 2015). Prior to the new terminology, studies showed that approximately 90% of patients with NMO and more than 50% of patients with NMOSD were positive for AQP4-IgG (Takahashi et al., 2007),(Waters et al., 2012), (Sato et al., 2014b). Before the introduction of the new diagnostic criteria for NMOSD in 2015, AQP4-IgG had also been reported in Asian patients with opticospinal MS (OSMS) and in patients with isolated optic neuritis (ON), isolated longitudinally extensive transverse myelitis (LETM), and rarely in patients with brainstem encephalitis, diencephalitis or posterior reversible encephalopathy (reviewed by Jarius and Wildemann) (Jarius and Wildemann, 2013). Because AQP4-IgG has been reported in 30–60% of OSMS patients, it was suggested that OSMS in the Asian population may be the same entity as NMO (Kira, 2011). Although AQP4-IgG was initially reported to be present in a high proportion of patients with NMOSD (Lennon et al., 2004) the sensitivity and specificity of the assay has varied in different populations and according to the type of assay used (Jarius and Wildemann, 2013). In a recent multicenter Australian and New Zealand survey of cases selected because they were considered to be 'highly suggestive of NMOSD', 177 cases were identified, of which 90% were seropositive based on immunofluoresence testing with a cerebellum substrate or a cell-based assay (CBA) in a subgroup of cases (Bukhari et al., 2017). However, the true prevalence of NMOSD in Australia and frequency of AQP4-IgG positive cases is not known.

In the present study we investigated the prevalence of AQP4-IgG positivity using a sensitive CBA in a consecutive diagnostic cohort of 196 sera or CSF samples from Western Australian patients being investigated for possible NMOSD referred to the State reference laboratory (PathWest Laboratory Medicine) over a 3 year period. A control group of 205 sera from patients with definite MS were also included in the study.

2. Methods

2.1. Study populations

We tested a total of 401 sera or CSF samples from two groups of patients. The first group was a diagnostic cohort comprising 196 consecutive serum or CSF (n = 9) samples from Western Australian patients with a presentation suggestive of NMOSD submitted to PathWest Laboratory Medicine for diagnostic AQP4-IgG testing during the period from June 2010 to November 2012. The referrals were from major hospitals and specialized medical centers in Western Australia. The serum and CSF samples had all previously been tested for AQP4-IgG reactivity by indirect immunofluorescence (IIF) using cryosections of primate cerebellum (INOVA, CA, USA) and monkey absorbed FITC-conjugated anti-human IgG (INOVA, CA, USA) and only 4 had tested AQP4-positive. The second group was a control MS cohort of 205 patients who all fulfilled the McDonald criteria for definite MS (Polman et al., 2005) and had previously also been tested for AQP4 immunoreactivity by IIF (Qiu et al., 2010a; Qiu et al., 2010b).

2.2. Ethics approval

The study was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee (HREC No: 2006-073 and QIA # 4105).

2.3. AQP4-IgG testing

To test for the presence of AQP4-IgG, we used the commercial cell-based BIOCHIP assay which employs fixed human embryonic kidney-293 (HEK-293) cells transfected with the M1 or M23 isoforms of AQP4 (Euroimmun, Luebeck, Germany). The AQP4-IgG test was performed following the manufacturer's protocol. Briefly, sera at a 1:10 dilution in phosphate buffered saline with tween (PBS-tween) were applied to the Biochip and incubated for 30 min at room temperature (RT) followed by incubation with fluorescein labeled anti-human IgG for 30 min at RT. A wash step with PBS-Tween was performed following all incubations. Slides were visualised with a Leica fluorescent microscope using an EL6000 metal halide light source. Two investigators (MJF-P and CB) scored the assays independently for antibody binding to the cytoplasm (0 to 3+) of the M1 and M23 transfected cells. A section of primate cerebellum was included on each biochip and staining of small vessels in the pia, subpial layer and Virchow-Robin spaces were recorded. All samples were blinded to the readers. Any assays with indeterminate staining were repeated.

3. Results

3.1. Frequency of AQP4-IgG

Among the diagnostic cohort of 196 consecutive patients, 5 (2.6%) were AQP4-IgG positive for M1- and M23-AQP4-IgG and 191 were AQP4-IgG negative. In the MS cohort there were no AQP4-IgG positive results. One patient with a previous diagnosis of OSMS was also included in the diagnostic cohort.

The 4 patients in the diagnostic cohort who had previously been reported as AQP4-IgG positive on the basis of tissue-based IIF were confirmed to be positive with the CBA. These included one patient who had previously been diagnosed as having OSMS. In addition, one other patient who had been negative on IIF testing, and had been diagnosed as MS, was shown to be AQP4-IgG positive with the CBA, leading to a revised diagnosis of NMOSD.

A small number of CSF samples ($n = 9$) were included in the study; 4 CSF samples were submitted with a matching serum sample collected with a maximum separation of 1 day between collections. The remaining CSF samples were submitted without a serum sample ($n = 3$), or with a separation of up to 3 months ($n = 2$). AQP4-IgG was not detected in any of the CSF samples.

3.2. Clinical and demographic features

When evaluating the ethnic background of the diagnostic cohort, we found that 179 patients were Caucasian, 16 Asian, 3 Maori and one of unknown ethnicity (Table 1). All 5 of the AQP4-IgG positive cases were females, with various ethnic backgrounds (2 Asian, 2 Caucasian, and 1 Maori), and their average age at onset was 49 years. All 5 fulfilled the

Table 1
Ethnic diversity and frequency of AQP4-positivity in the diagnostic cohort.

Ethnic background	Number (n)	Percentage (%)	AQP4-IgG positive (n)	AQP4-IgG positive (%)
Caucasian	176	89.8	2	1.1
Asian	16	8.2	2	12.5
Maori	3	1.5	1	33.3
Unknown	1	0.5	0	NA