

## Low sensitivity of anti-aquaporin-4 antibody in multiple sclerosis, longitudinally extensive spinal cord lesions and neuromyelitis optica in Australians

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*Background and Objective:* Multiple Sclerosis (MS) is the most common neurological disease of young adults in Western countries. The spectrum of demyelinating disease in Western countries is characterised by predominance of conventional MS and small proportion of neuromyelitis optica (NMO). NMO is similar to optic-spinal MS reported in Asia in many aspects. It has been argued that relapsing NMO and optic-spinal MS is different from conventional MS with clinical and laboratory features such as female predominance, low frequencies of oligoclonal IgG bands, longitudinally extensive (>3 vertebral segments) spinal cord lesions (LESCLs), CSF pleocytosis, and poor clinical prognosis. Moreover, a recently identified serum autoantibody, so called NMO-IgG, has been proposed as a biomarker to distinguish NMO from conventional MS. The target antigen of NMO-IgG was identified as aquaporin-4 (AQP4) water channel protein.<sup>1</sup> An anti-AQP4 antibody assay using human AQP4-transfected cells appeared more sensitive than the original NMO-IgG assay.<sup>2</sup> The presence of anti-AQP4 antibody seemed to be associated with LESCLs and exacerbation of disease.<sup>3</sup> Our study aimed to investigate the prevalence of anti-AQP4 antibody in conventional MS, NMO and LESCL patients from a Western Australian MS cohort.

*Methods:* A cohort of 842 MS patients seen by two neurologists were reviewed and a summary of their historical, clinical, laboratory features and MRI findings were described in the previous report. According to the McDonald criteria<sup>4</sup>, 703 patients were diagnosed as having clinically definite or possible MS. MS with lesions confined to the optic nerve and spinal cord, with no evidence of lesions in the cerebrum or cerebellum, or minor lesions not satisfying the Barkhof diagnostic criteria were classified as optic-spinal MS. Patients with minor brainstem signs were also classified as optic-spinal MS. Invitation letters were sent out to all optic-spinal MS, primary progressive MS, LESCL patients and some conventional MS patients. Blood samples were collected from those who were willing to participate in this study and DNA samples for further HLA genotyping were stored. The anti-AQP4 antibody test was kindly performed by Dr Takuya Matsushita at Kyushu University, using GFP-AQP4 expressing cells. We also included the results of three NMO cases (one case also had AQP4 testing) whose serum was previously tested for NMO-IgG at the Mayo clinic in this study. All statistical analysis (means, standard deviation), significance of group differences ( $\chi^2$  and T-tests) were performed using the SPSS V12.0 for windows. P values of <0.05 were considered statistically significant.

*Results:* Our study showed a low sensitivity of anti-AQP4 antibody in this predominantly Caucasian MS population, with only two cases (2%) found to be seropositive in 96 samples. The NMO-IgG test done at Mayo Clinic for three NMO patients were all negative, among them one was also anti-AQP4 antibody negative. The other two cases were deceased.

There was one anti-AQP4 antibody positive case (1.9%) among the 52 relapsing MS cases, and no anti-AQP4 antibody positive case was found in the group of primary progressive MS. Among the 18 optic-spinal MS patients only one case was anti-AQP4 antibody positive (5.6%). There were 14 LESCL cases in this MS cohort with one LESCL presenting positive anti-AQP4 antibody (7.1%). The clinical features of this cohort of MS patients are shown in Table 1. The details of the two cases with positive anti-AQP4 antibody are as follows.

**Table 1: Clinical characteristics of MS patients in the Western Australian cohort**

	Conventional MS		Optic-spinal MS (n=18)
	Relapsing-remitting MS (n=52)	Primary progressive MS (n=23)	
Female/Male	3.7	1.1*	0.8*
Onset age ( $\pm$ SD) (years)	33.7 + 8.9	44.1 + 10.0	12.2 + 9.8
Percent anti-AQP4 Ab Positive (%)	1.9 (1/52)	0	5.6 (1/18)
Percent oligoclonal bands positive	68.6 (24/35)	75 (6/8)	44.4 (4/9)
Percent with LESCLs	12.5 (6/48)	5.3 (1/19)	22.2 (4/18)

\* P<0.05, \*\*P<0.01, Value is significant in comparison with conventional MS

*Case 1.* This is a woman with classical relapsing remitting MS, now secondary progressive. First symptom onset at the age of 39 in 1982, with duration of disease of 25 years. Brain MRI showed typical lesions meeting Barkhof diagnostic criteria and spine MRI showed multiple short lesions. CSF Oligoclonal bands were negative at initial diagnosis.

*Case 2.* This 37 year old woman initially presented with typical MS symptoms and classical brain MRI in June 2006, with the subsequent onset of severe myelopathy whilst on IFN-beta therapy. MRI spine showed LESCL of 6 vertebral segments from T2-T9. The initial clinical presentation was consistent with conventional MS, but was revised to NMO following the development of the spinal lesion.

*Conclusion:* Our study showed a very low rate of anti-AQP4 antibody in Caucasian MS patients. Moreover, there was only one positive anti-AQP4 antibody in 18 NMO or optic-spinal MS cases. Although one positive case was associated with LESCLs, the sensitivity of anti-AQP4 antibody to LESCLs was still extremely low in comparison with other studies.<sup>2,5</sup> These findings are in direct variance with results from other patient populations tested in the same way. This difference indicates the need for further study on the clinical utility of AQP4 serology. We are preparing a comprehensive serological and immunogenetic examination of 900 patients.

## References

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