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ABSTRACTS OF PRESENTATIONS RELATED TO ANTI-AQUAPORIN-4 ANTIBODY

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Anti-aquaporin-4 antibody in a large population of Japanese multiple sclerosis patients

Keiko TANAKA

Department of Neurology, Brain Research Institute, Niigata University, Japan

Background and Objective: Multiple sclerosis (MS) could be classified clinically into two forms – conventional and optic-spinal MS. About 20% of Japanese MS patients have optic-spinal MS. Neuromyelitis optica (NMO) or recurrent Devic disease is thought to be more common among Asians and Africans, have higher female preponderance, manifests as severe optic nerve and spinal cord lesions, have high relapse rate, fewer ovoid lesions in brain magnetic resonance imaging (MRI) and causes long spinal cord lesions (more than 3 vertebral segments in length). Cerebrospinal fluid oligoclonal bands are rarely positive and various autoantibodies (such as ANA, anti-SSA and anti-SSB) are frequently found to be positive. Pathologically, NMO lesions show swelling, softening and vacuolation macroscopically. Microscopically, there is necrosis in the gray and white matter, with neutrophil and eosinophil infiltration. The blood vessels are hyalinated, and there are IgG and activated complement deposition. The C9neo antigen is expressed on the outer surface of thickened vessel wall with IgM deposition within the wall. Optic-spinal MS on the other hand, is a clinical and not a pathological diagnosis. This incurs several problems. Firstly, there is an overlap with the diagnosis of NMO; and secondly, the diagnosis could change to classical MS if the patient develops cerebral lesion later. To investigate the use of anti-aquaporin-4 (AQP4) antibody in the diagnosis of NMO and optic-spinal MS we developed our own anti-AQP4 antibody assay and tested the presence of the antibody in a large population of Japanese MS patients.

Methods: For the detection of NMO-IgG, we use similar methodology as Lennon *et al.*[1], except that we use cryostat section of rat and mouse cerebrum and cerebellum, and fixed the section with cold acetone for 2 minutes. To produce AQP4 transfected cells for the anti-AQP4 antibody assay, we cloned human AQP4-cDNA from human brain total RNA. The cDNA was then inserted into an expression vector, and HEK 293 cells were transfected with this vector. The cells were then fixed with 4% paraformaldehyde/PBS, incubated with the patients' sera or cerebrospinal fluid, and finally with FITC-conjugated anti-human IgG.

Results: We compared the immunofluorescence detection of AQP4 transfected HEK 293 cells with indirect immunofluorescence staining of rodent brain tissue and found that the test yield comparable results.

We received 1,110 clinical samples (508 were optic-spinal MS) from hospitals throughout Japan. Of these, 140 samples from 115 patients (102 or 88.7% female) were tested positive. The mean age at examination was 50.2 $\pm$ 15.5 years and the mean onset age was 43.9 $\pm$ 15.9 years. The EDSS score was 5.6 $\pm$ 2.3. Clinically, the initial lesions were in the spinal cord in 33 patients, optic nerves in 32, and 5 each in the cerebrum and brainstem. MRI showed long cord lesions in 95 patients (84%) and segmental atrophy in 23 patients. Cerebral lesions were seen in 74 patients (68%) and brainstem in 8 (11%). Forty-nine (46.2%) patients were blind. Autoantibodies seen in other autoimmune diseases were found in 36 patients (43.9%). Oligoclonal bands were positive in 12 patients (12.8%). The mean

number of exacerbation was 3.6 per annum. Patients with anti-AQP4 antibody were more likely to be female and blind, and had more relapses yearly. Close to 20% of samples from optic-spinal MS patients with long cord lesions were tested negative. These patients tended to showed mild visual symptoms and had low exacerbation rates. There were 5 patients (all female) who were tested positive for anti-AQP4 antibody but did not have long cord lesions. Their mean age was 51.6 $\pm$ 31.3 years, their onset age was 49.6 $\pm$ 32.5 years. The initial lesion was in the optic nerve in 4; 3 out of 4 were blind, and an equal proportion had other autoantibodies. The antibody titre tended to increase during exacerbation and reduce during remission. The titre varied from 1:1,600 to 1:51,200 in the sera and 0 to 1:40 in the cerebrospinal fluid in the same patient. HEK cells expressing AQP4/GFP were also shown to have increased water permeability when incubated with anti-AQP4 IgG.

Conclusion: We found that the anti-AQP4 antibody is an excellent marker for both NMO and optic-spinal MS patients with long spinal cord lesions. Among the seropositive patients, there is female preponderance; the onset age is late, and the patients tended to have severe optic nerve or spinal cord lesions with cord lesions longer than 3 vertebral segments. Recurrence was frequent and there was high frequency of cerebral lesions on brain MRI and low frequency of oligoclonal bands in cerebrospinal fluid.

The anti-AQP4 antibody might be directly related to the pathogenesis of the disease as the antibody titres related closely to the disease activity. The lesions involved were also closely related to the areas of abundant AQP4 water channel. Immune complexes and activated complement were found to be deposited in the lesions, and early lesions in NMO were found to have profound loss of AQP4 channel. Anti-AQP4 IgG was also found to alter cellular permeability to water in AQP4-expressing cells.

Reference

1. Lennon VA, Wingerchuk DM, Kryzer TJ, *et al*. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* 2004; 364(9451): 2106-12.