

Title: Myelin and Astrocyte Injury in Neuroinflammatory Disorders with MOG or aquaporin-4

Antibodies

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ABSTRACT (MAX = 250 words)

Objective: To compare myelin and astrocyte injury in patients with antibodies against myelin oligodendrocyte glycoprotein (anti-MOG) or aquaporin-4 (anti-AQP4), and multiple sclerosis (MS).

Methods: Myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP) levels were measured in the cerebral spinal fluid (CSF) from anti-MOG⁺ or anti-AQP4⁺ patients tested in both sera and CSF by cell-based assays with live transfected cells.

Results: In total, 75.6% (68/90) of the patients were positive for either anti-MOG or anti-AQP4 antibodies in both serum and CSF; 74.2% (23/31) were anti-MOG⁺, and 76.3% (45/59) were anti-AQP4⁺. No patients were only CSF positive or were positive for both anti-MOG and anti-AQP4 antibodies, and none of the MS patients or controls had these autoantibodies in the serum or CSF. MBP levels were elevated in the anti-MOG⁺ cases compared to the multiple sclerosis (MS) patients, and the levels found were similar between anti-MOG⁺ cases and anti-AQP4⁺ neuromyelitis optica spectrum disorder (NMOSD) cases. Meanwhile, GFAP was only elevated in the anti-AQP4⁺ NMOSD. Moreover, CSF pleocytosis, high protein levels, and oligoclonal IgG band negativity distinguished the anti-MOG⁺ cases from MS patients.

Conclusions: Myelin injury was more severe in the anti-MOG⁺ cases than in the MS cases, and anti-MOG⁺ cases have differences in the CSF characteristics compared to MS. GFAP elevation in anti-AQP4⁺ cases was absent in anti-MOG⁺ patients (even in cases with NMOSD phenotype), indicating

that immune-mediated astrocytopathy is unique to anti-AQP4+ patients. Our study suggests that anti-MOG+ cases are distinct from MS and anti-AQP4+ NMOSD.

BACKGROUND

Acquired inflammatory central nervous system (CNS) disorders include multiple sclerosis (MS), neuromyelitis optica spectrum disorders (NMOSD), acute disseminated encephalomyelitis (ADEM), optic neuritis (ON), transverse myelitis (TM), and other less prevalent disorders.¹ Differential diagnosis between these entities may be challenging in some cases due to overlapping clinical characteristics. Antibodies against aquaporin-4 (anti-AQP4) suggest the diagnosis of NMOSD.^{2,3} Several studies have demonstrated the pathogenicity of anti-AQP4 antibody binding to astrocytes, causing severe immune-mediated CNS tissue damage.³ Moreover, during NMOSD attacks, cerebrospinal fluid (CSF) anti-AQP4 antibody titers correlated well with CSF levels of glial fibrillary acidic protein (GFAP), indicating astrocyte injury.⁴ However, some patients who are clinically diagnosed with NMOSD are anti-AQP4-seronegative despite the use of highly sensitive cell-based assays (CBAs); therefore, other CNS molecules may be implicated as autoimmune targets in such cases. Recently, we and others have reported the presence of conformational-sensitive antibodies against myelin oligodendrocyte glycoprotein (anti-MOG) in a portion of anti-AQP4-seronegative NMOSD, ADEM and ON cases, but these antibodies are not usually found in typical MS using highly specific CBAs.⁵ We have also reported an elevation of CSF myelin basic protein (MBP) without detectable CSF-GFAP, suggesting myelin damage without astrocyte injury in an anti-MOG+, anti-AQP4-negative patient with definite NMO.⁶

Thus, it is interesting to study how anti-MOG⁺ cases pathologically and immunologically differ from anti-AQP4⁺ cases and MS cases. In this multicenter international collaborative study, we evaluated anti-MOG and anti-AQP4 antibodies in the CSF and compared markers of myelin and astrocyte damage in the CSF as well as other CSF parameters between the three groups.

PATIENTS AND METHODS

We enrolled a total of 90 patients diagnosed with acquired inflammatory CNS disorders and serum positivity for anti-MOG or anti-AQP4 antibodies by CBAs from Japan, Brazil, Republic of Korea, Spain, France, Austria, and Thailand (see study flowchart in Figure 1). For the European countries, serum antibody testing was performed in each country. We also included CSF samples from 40 relapsing-remitting MS patients collected during relapses who fulfilled the 2010 McDonald criteria⁷ as a control group. We only included cases for which a CSF sample was stored at each center, and all samples were shipped in dry ice to Sendai, Japan.

All of the CSF samples were analyzed at Tohoku University to detect anti-AQP4 and anti-MOG antibodies using CBAs with M23-AQP4 or full-length MOG-transfected HEK293 cells as previously described.⁸ In those samples with confirmed CSF positivity for anti-AQP4 or anti-MOG antibodies, astrocyte and myelin damage were evaluated by measuring CSF levels of GFAP and MBP, respectively, using commercially available enzyme-linked immunosorbent assays (ELISAs) from Cosmic Corporation

(Tokyo, Japan) and SPI bio (Montigny le Bretonneux, France).⁹

We compared the clinical and laboratory data of the anti-MOG⁺, anti-AQP4⁺ and MS groups using nonparametric tests (Wilcoxon tests) for the continuous variables and Fisher's exact test for the categorical data. Spearman's correlation coefficient rank test was used to evaluate the correlation between CSF cell counts, total protein, antibody titers, and CSF MBP and GFAP levels in samples collected within 30 days from symptom onset and before any acute treatment. Two-tailed $p < 0.05$ was considered to be significant.

This study was approved by the ethics committee of each center and was conducted in accordance with internationally recognized ethical standards. All of the study participants provided written informed consent.

RESULTS

In total, 75.6% (68/90) of the patients were positive for either anti-MOG or anti-AQP4 antibodies in both serum and CSF; 74.2% (23/31) were anti-MOG⁺, and 76.3% (45/59) were anti-AQP4⁺. No patients were only CSF positive or were positive for both anti-MOG and anti-AQP4 antibodies, and none of the 40 MS patients had these autoantibodies in serum or CSF. The clinical characteristics are summarized in Table 1.

Compared to the anti-AQP4⁺ group, the anti-MOG⁺ group exhibited a younger median age at CSF collection ($p < 0.0001$), a higher proportion of males ($p = 0.0205$), a greater frequency of ON attacks ($p <$

0.0001), and a reduced frequency of the NMO phenotype ($p = 0.0015$); however, there was no difference in the frequency of TM ($p = 0.3587$). Moreover, the anti-MOG⁺ patients were significantly younger ($p = 0.0283$), and the proportion of males was higher ($p = 0.0334$) than in the MS group.

With regard to the routine CSF analysis, CSF cell counts were elevated in the anti-MOG⁺ group compared to the anti-AQP4⁺ ($p = 0.0002$) and MS ($p < 0.0001$) groups. CSF protein levels were similar in the anti-MOG⁺ and anti-AQP4⁺ groups ($p = 0.2341$) but were higher than in the MS group ($p = 0.0047$). CSF oligoclonal immunoglobulin G (IgG) bands (OCBs) were positive in a single case (5.3%) of the anti-MOG⁺ cases and in 11.4% of the anti-AQP4⁺ cases; in contrast, 76.7% of the MS patients exhibited CSF-OCB positivity (anti-MOG⁺ vs. MS, $p < 0.001$ and anti-AQP4⁺ vs. MS, $p < 0.0001$).

In the patients with autoantibody positivity in both serum and CSF, the time from symptom onset to CSF collection did not differ significantly between the anti-AQP4⁺ (36.7 ± 72.5 days) and anti-MOG⁺ samples (13.81 ± 16.95 days, $p = 0.3513$). The remarkable elevation of GFAP in the anti-AQP4⁺ CSF samples (9101 ± 29835 ng/ml) was not observed in the anti-MOG⁺ CSF samples (1.7 ± 3.1 ng/ml, $p < 0.0001$) or in the CSF samples from the MS patients (0.9 ± 0.7 ng/ml, $p < 0.0001$) (Figure 2). In contrast, elevated MBP was observed in the anti-MOG⁺ CSF (804.6 ± 1029 pg/ml), and these MBP levels were similar to those observed in the anti-AQP4⁺ CSF (573 ± 668.7 pg/ml) ($p = 0.7066$). Both of these groups exhibited significantly higher MBP levels than the MS group (296.5 ± 423.3 pg/ml) ($p = 0.0382$ and $p = 0.0058$, anti-MOG⁺ vs. MS and anti-AQP4⁺ vs. MS, respectively).

In the 30 anti-AQP4+ CSF samples collected < 30 days from symptom onset and before any treatment, there was good correlation between GFAP levels and CSF cell counts ($r = 0.5963$, $p = 0.001$), protein ($r = 0.6195$, $p = 0.0004$), and anti-AQP4 titers ($r = 0.4401$, $p = 0.0149$), but MBP levels were not correlated with any of these parameters. In the anti-MOG+ CSF ($n = 18$) that was collected within 30 days from symptom onset and before any treatment, MBP levels were elevated ($916 \pm 1,097$ pg/ml), but they did not correlate with CSF cell counts, protein, or MOG titers.

DISCUSSION

In this international multicenter study, we described CSF findings for anti-MOG+, anti-AQP4+ and MS patients. Astrocyte injury was evident in the anti-AQP4+ CSF but was not observed in CSF samples from the anti-MOG+ or MS patients. Anti-AQP4 seropositivity is important for the diagnosis of NMOSD,¹⁰ and experimental animal models can reproduce NMOSD-like pathology.¹¹ Moreover, we previously reported that anti-AQP4+ CSF titers are strongly correlated with CSF-GFAP and pro-inflammatory cytokine levels, cell counts and protein.⁴ Taken together, these results indicate that the injury of astrocytes and other CNS cells is closely related to anti-AQP4 antibodies in the CNS.

Significantly higher CSF-MBP levels were observed in the anti-MOG+ and anti-AQP4+ cases than in MS, but the pathomechanisms underlying myelin damage in anti-AQP4+ NMOSD and anti-MOG-associated neurologic diseases may differ. While myelin damage in anti-AQP4+ NMOSD seems to be secondary to

anti-AQP4 antibody-induced astrocyte injury,¹² perhaps anti-MOG antibodies cause myelin damage by binding directly to MOG.¹³ Brain lesions from of an anti-MOG+ patient with encephalomyelitis demonstrated inflammatory demyelination with deposition of immunoglobulins and complement.¹⁴ The lesions were infiltrated with T cells, macrophages and microglia, but lacked the loss of AQP4, a pathological hallmark of anti-AQP4+ NMOSD. Moreover, we recently reported the elevation of CSF interleukin-6 in one anti-MOG+ patient with NMO phenotype, so this multifunctional cytokine may induce the recruitment of immune cells (MOG-reactive T cells and B cells, macrophages, etc.) and the release/synthesis of co-factors that promote demyelination (e.g., proinflammatory cytokines and chemokines as well as complement).¹⁵ Further studies are required to elucidate the involvement of cellular immunity in the myelin injury associated with anti-MOG antibodies.

The retrospective design of the present study could result in some heterogeneity in the CSF samples, but we confirmed the anti-AQP4 and anti-MOG positivity of the CSF samples using in-house CBAs. We confirmed the elevation of CSF-GFAP levels in the anti-AQP4+ samples, but the CSF-MBP levels varied more compared to CSF-GFAP levels,¹⁶ which may explain why CSF-MBP levels did not correlate with anti-AQP4 or anti-MOG titers or cell counts and protein levels. Further studies are needed to identify more sensitive CSF biomarkers of myelin damage.

In conclusion, our CSF study demonstrated that GFAP elevation in anti-AQP4+ cases was absent in anti-MOG-associated neurologic diseases (including cases with NMOSD phenotype), indicating that

immune-mediated astrocytopathy is unique to anti-AQP4+ patients. Moreover, myelin injury was more severe in the anti-MOG+ cases than in the MS cases, and the anti-MOG+ cases exhibited some unique CSF characteristics and clinical and MRI features, suggesting that anti-MOG+ cases are distinct from MS and anti-AQP4+ NMOSD.⁷⁻⁹ Future studies may further clarify the pathogenesis of this anti-myelin autoantibody-associated disease, leading to better diagnosis and novel therapeutic strategies.

AUTHORSHIP

KK = study conception, data analysis and draft the manuscript; DKS = study conception, data analysis and draft the manuscript; IN = study conception, data analysis and reviewed the manuscript; SN = data acquisition and reviewed the manuscript; ST = data acquisition and reviewed the manuscript; RM = study conception, data analysis and draft the manuscript; JWH = data acquisition and reviewed the manuscript; LMO = data acquisition and reviewed the manuscript; MR = study conception, data analysis and draft the manuscript; TSH = data acquisition and reviewed the manuscript; MS = data acquisition and reviewed the manuscript; SS = data acquisition and reviewed the manuscript; PJW = study conception, data analysis and draft the manuscript; KaK = data acquisition and reviewed the manuscript; TA = data acquisition and reviewed the manuscript; HK = data acquisition and reviewed the manuscript; TM = data acquisition and reviewed the manuscript; NP = data acquisition and reviewed the manuscript; TB = data acquisition and reviewed the manuscript; AS = data acquisition and reviewed the manuscript; HJK = study conception, data analysis and reviewed the manuscript; KN = study conception and reviewed the manuscript; DC = study conception, data analysis and reviewed the manuscript; KF = study conception, data analysis and draft the manuscript; MA = study conception and reviewed the manuscript

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TABLE 1

| | Anti-MOG+ | Anti-AQP4+ | Multiple Sclerosis |
|-------------------------------------|-----------------|-----------------|--------------------|
| | (n = 31) | (n = 59) | (n = 40) |
| Age at CSF collection | 28 (2 - 71)** | 49 (15 - 79) | 34 (19 - 55) |
| Gender (Male:Female) | 13:18* | 10:49 | 7:33 |
| Clinical diagnosis | | | |
| <i>NMO</i> | 6 (19.3%)* | 33 (55.9%) | |
| <i>Transverse Myelitis</i> | 9 (29.0%) | 24 (40.7%) | |
| <i>Optic Neuritis</i> | 11 (35.4%)* | 2 (3.3%) | |
| <i>ADEM</i> | 5 (16.1%) | 0 | |
| CSF findings | | | |
| <i>Cell count (/mm³)</i> | 12 (0 - 247) ** | 3 (0 - 410) | 2 (0 - 18) |
| <i>Protein (mg/dl)</i> | 40(14 - 417) # | 51 (21 - 229) # | 25 (12 - 77) |
| <i>OCB positivity</i> | 1/19 (5.8%)# | 5/43 (11.6%)# | 23/30 (76.7%) |

Abbreviations: MOG = myelin oligodendrocyte glycoprotein; AQP4 = aquaporin-4; CSF = cerebrospinal fluid; NMO = neuromyelitis optica; ADEM = acute disseminated encephalomyelitis; OCB = oligoclonal IgG band.

$p < 0.05$: * vs. anti-AQP4+, ** vs. anti-AQP4+ and MS, # vs. MS.

FIGURE LEGENDS

Figure 1

Study flowchart. In total, 90 cerebrospinal fluid (CSF) samples from Japan, Brazil, Republic of Korea, France, Spain, Austria, and Thailand were analyzed at Tohoku University. The European patients were tested for serum antibodies against myelin oligodendrocyte glycoprotein (MOG) and aquaporin-4 (AQP4) in each country using cell-based assays; the sera of the patients from the other countries were tested at Tohoku University. Only patients with antibody positivity in both serum and CSF were included in the comparison analysis, and only samples collected < 30 days before the onset of symptoms and prior to treatment were included in the correlation analysis.

Figure 2

(A) Glial fibrillary acidic protein (GFAP) in the cerebrospinal fluid (CSF) of anti-aquaporin-4 (AQP4)-positive, anti-myelin oligodendrocyte glycoprotein (MOG)-positive, and multiple sclerosis (MS) patients. (B) Myelin basic protein (MBP) in the CSF of the anti-AQP4+, anti-MOG+, and MS patients. Open circles = transverse myelitis, closed circles = optic neuritis, triangles = acute disseminated encephalomyelitis, and squares = multiple sclerosis.