Markedly Elevated Soluble Intercellular Adhesion Molecule 1, Soluble Vascular Cell Adhesion Molecule 1 Levels, and Blood-Brain Barrier Breakdown in Neuromyelitis Optica

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Objective: To evaluate the degree of blood-brain barrier disruption in patients with neuromyelitis optica (NMO) and to clarify whether the levels of soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1) in patients with NMO can be useful biomarkers for blood-brain barrier breakdown.

Design: Descriptive historical cohort.

Setting: Department of Neurology, Graduate School of Medicine, Chiba University.

Patients: The levels of sICAM-1 and sVCAM-1 in 25 patients with NMO, 21 patients with multiple sclerosis, and 20 patients with other noninflammatory neurologic disorders in the serum and cerebrospinal fluid (CSF) were measured using a multiplexed fluorescent magnetic bead-based immunoassay.

Main Outcome Measures: Levels of the soluble adhesion molecules in serum and CSF and their associations with blood-brain barrier disruption.

Results: The CSF levels of sICAM-1 and sVCAM-1 increased in patients with NMO compared with patients with multiple sclerosis and other noninflammatory neurologic disorders (P < .001), and serum levels of sICAM-1 increased in patients with NMO compared with healthy control individuals (P = .003). The CSF sICAM-1 levels from patients with NMO were correlated with the albumin quotient (P = .02) and the presence of lesions detected via gadolinium-enhanced magnetic resonance imaging.

Conclusions: Severe blood-brain barrier breakdown occurs in patients with NMO. Measuring adhesion molecules is useful to evaluate this barrier disruption.

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DHESION MOLECULES, SUCH as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), play an important role in the migration of activated peripheral blood T cells to the central nervous system (CNS). Soluble forms of ICAM-1 (sICAM-1) and VCAM-1 (sVCAM-1) have been suggested to play a major role in the regulation of the blood-brain barrier in patients with multiple sclerosis (MS). Neurmyelitis optica (NMO) is an autoimmune inflammatory disorder of the CNS that predominantly affects the optic nerves and the spinal cord and is presumed to be a different disease than MS. An elevation of the levels of serum antibody against aquaporin 4 (AQP4), which is densely expressed in astrocytic foot processes, has been described in patients with NMO. However, disruption of the blood-brain barrier would be necessary to allow this autoantibody to enter the CNS. In this study, we measured the levels of sICAM-1 and sVCAM-1 in serum and cerebrospinal fluid (CSF) in patients with NMO to detect blood-brain barrier disruption and to further elucidate the pathogenesis of NMO.

METHODS

STUDY PARTICIPANTS

We assayed serum and CSF samples obtained simultaneously during a relapse before initiation of treatment from 25 consecutive patients with NMO who fulfilled the criteria outlined by Wingerchuk et al (all women; median age, 50.4 years), 21 patients with relapsing-remitting MS (15 women and 6 men; median age, 34.3 years) who fulfilled the revised McDonald criteria, and 20 patients with other noninflammatory neurologic disorders (ONNDs; amyotrophic lateral sclerosis, 9; spinocerebellar degeneration, 9; progressive supranuclear palsy, 2; a total of 8 women and 12 men; median age, 57.1 years). In addition, 17 individuals (15 women and 2 men; median age, 32.0 years) served as healthy controls (HCs). All samples were immediately stored at
Scleroses; NA, not applicable; NMO, neuromyelitis optica; ONNDs, other noninflammatory neurologic diseases.

Higher in patients with NMO (P<.001) in those with MS or ONNDs (P=.003). Dashed lines indicate the mean levels in each group. The albumin quotient (ie, the CSF–serum albumin quotient) of the total patients, 18 with NMO and 15 with MS underwent GdMRI when serum and cerebrospinal fluid were sampled.

Table. Clinical and Laboratory Findings of Patients With NMO, MS, and ONNDs

<table>
<thead>
<tr>
<th>Finding</th>
<th>NMO (n=25)</th>
<th>MS (n=21)</th>
<th>ONNDs (n=20)</th>
<th>NMO vs MS</th>
<th>NMO vs ONNDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDSS score, median (range)</td>
<td>7.0 (2.0-9.0)</td>
<td>3.0 (1.0-7.0)</td>
<td>NA</td>
<td>&lt;.001</td>
<td>NA</td>
</tr>
<tr>
<td>CSF cell count, mean (SD), /µL</td>
<td>18.4 (27.5)</td>
<td>8.2 (10.0)</td>
<td>1.0 (1.0)</td>
<td>.21</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CSF protein, mean (SD), mg/dL</td>
<td>66.3 (49.4)</td>
<td>33.1 (9.7)</td>
<td>34.5 (12.8)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Albumin quotient ×10^4, mean (SD)</td>
<td>1.1 (1.0)</td>
<td>0.5 (0.1)</td>
<td>0.4 (0.1)</td>
<td>&lt;.001</td>
<td>.003</td>
</tr>
<tr>
<td>Interval between clinical relapse and sampling, median (range), d</td>
<td>3.0 (1.0-29.0)</td>
<td>14.0 (1.0-29.0)</td>
<td>NA</td>
<td>&lt;.001</td>
<td>NA</td>
</tr>
<tr>
<td>Positive for anti–aquaporin 4 antibody, No. (%)</td>
<td>19/25 (76)</td>
<td>0</td>
<td>0</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Length of spinal cord lesion, vertebral segments, median (range)</td>
<td>5.0 (2.0-10.0)</td>
<td>1.0 (0-5.0)</td>
<td>NA</td>
<td>&lt;.001</td>
<td>NA</td>
</tr>
<tr>
<td>Lesion at relapse, No. (%)</td>
<td>24/25 (96)</td>
<td>1/21 (5)</td>
<td>NA</td>
<td>&lt;.001</td>
<td>NA</td>
</tr>
</tbody>
</table>

Lesion detected via GdMRI:
- Brain: 4/25 (16) vs 20/21 (95), P<.001
- Spinal cord: 20/25 (80) vs 13/21 (62), P=.15
- Optic nerve: 1/25 (4) vs 1/21 (5), P=.71
- Other: 1/25 (4) vs 1/21 (5), P=.23

Abbreviations: CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; GdMRI, gadolinium-enhanced magnetic resonance imaging; MS, multiple sclerosis; NA, not applicable; NMO, neuromyelitis optica; ONNDs, other noninflammatory neurologic diseases.

We also analyzed the correlations between the CSF and serum sICAM-1 and sVCAM-1 levels and the clinical and laboratory findings, such as the Expanded Disability Status Scale (EDSS) score, CSF cell count, CSF protein level, albumin quotient, optical density of the serum anti-AQP4 antibody at 450 nm, maximum length of the spinal cord lesion via T2-weighted magnetic resonance imaging (MRI), location of the lesion at relapse, and positivity of the lesion via gadolinium-enhanced MRI (GdMRI) in patients with NMO.

Ethics approval was granted by the Ethics Committee of Chiba University School of Medicine, Chiba, Japan. All the study participants gave informed consent for their participation.

**OUTCOME MEASURES**

The serum and CSF samples were centrifuged, and the supernatants were collected. The samples were diluted 200-fold in serum and 24-fold in CSF and simultaneously analyzed for sICAM-1 and sVCAM-1 using a multiplexed fluorescent magnetic bead-based immunoassay (Bio-Rad Laboratories Inc, Hercules, California), according to the manufacturer’s instructions. The sICAM-1 and sVCAM-1 concentrations were calculated by referring to a standard curve for each set of adhesion molecules derived from standard assays.

**DATA ANALYSIS**

The groups were compared using the Fisher exact test for categorical outcomes and the Mann-Whitney test for continuous variables, as appropriate. The Spearman rank correlation coefficient was used to test the associations. All comparisons were planned, and tests were 2-sided. All statistical analyses were performed using SPSS statistical software, version 16.0J (SPSS Japan Inc, Tokyo, Japan).

**RESULTS**

**CLINICAL CHARACTERISTICS**

Clinical profiles of patients with NMO, MS, and ONNDs are summarized in the Table. The mean (SD) CSF cell counts are 18.4 (27.5)/µL, CSF protein is 66.3 (49.4) mg/dL, and so forth.
and albumin quotient $\times 10^2$ (1.11 [1.02]) in patients with NMO were significantly higher than those in patients with MS and ONNDs ($P = .21$ and $P < .001$ for CSF cell counts, $P < .001$ and $P < .001$ for CSF protein, and $P = .003$ for albumin quotient, respectively). The EDSS score (median, 5.0), length of the spinal cord lesion via T2-weighted MRI (median, 5.0 vertebral segments), number of patients with 3 or more vertebral-segment spinal cord lesions (96%), and positivity of the anti-AQP4 antibody (76%) in patients with NMO were higher than those in patients with MS ($P < .001$ for each). The interval between clinical relapse and sampling was significantly shorter in patients with NMO (median, 3.0 days) than in patients with MS (median, 14.0 days). However, no correlation was observed between the interval and levels of CSF adhesion molecules in patients with NMO (sICAM-1: $r = -0.03$, $P = .87$; sVCAM-1: $r = -0.19$, $P = .30$) or those with MS (sICAM-1: $r = 0.09$, $P = .72$; sVCAM-1: $r = 0.15$, $P = .52$). Lesions at relapse and positive results for lesions detected via GdMRI in patients with NMO were not significantly different from those in patients with MS.

**sICAM-1 AND sVCAM-1 LEVELS**

The mean CSF and serum values of sICAM-1 and sVCAM-1 from HCs and patients with NMO, MS, and ONNDs are summarized in Figure 1. The CSF levels of sICAM-1 and sVCAM-1 were significantly higher in patients with NMO than in patients with MS and ONNDs ($P < .001$). The serum sICAM-1 levels were higher in patients with NMO ($P = .003$) and patients with ONNDs ($P = .007$), and the same tendency was seen in patients with MS ($P = .048$) compared with HCs.

**CORRELATIONS BETWEEN CLINICAL AND LABORATORY FINDINGS AND sICAM-1 AND sVCAM-1 LEVELS IN PATIENTS WITH NMO**

The CSF sICAM-1 levels significantly correlated with the albumin quotient ($r = 0.596$, $P = .02$), CSF cell counts ($r = 0.581$, $P = .002$), and CSF protein level ($r = 0.710$, $P < .001$), but CSF sVCAM-1 levels correlated significantly only with CSF protein ($r = 0.550$, $P = .004$) and not with the albumin quotient ($r = 0.443$, $P = .10$) or CSF cell counts ($r = 0.336$, $P = .10$) in patients with NMO (Figure 2). The optical density of the serum anti-AQP4 antibody at 450 nm, EDSS score, and length of the spinal cord lesion were not significantly correlated with sICAM-1 and sVCAM-1 levels in patients with NMO, and serum adhesion molecules were not significantly associated with clinical and laboratory findings. The CSF adhesion molecule levels of patients with NMO who had lesions detected via GdMRI (n=14) (sICAM-1: 4341.8 [3681.5] pg/mL; sVCAM-1: 15819.2 [5009.3] pg/mL) were much higher than without lesions via GdMRI (n=4) (sICAM-1: 2145.9 [1080.6] pg/mL; sVCAM-1: 11025.2 [3708.1] pg/mL) (Figure 3). No significant differences were found in the adhesion molecule levels related to location of the lesion at relapse in the patients with NMO.

**COMMENT**

The blood-brain barrier is formed by capillary endothelial cells, surrounded by the basal lamina and astrocytic endfeet; it regulates the interaction between the immune sys-
Critical revision of the manuscript for important intellectual content: Uzawa, Mori, and Kuwabara.
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Figure 3. Cerebrospinal fluid (CSF) soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1) levels in patients with neuromyelitis optica (NMO) with or without lesions detected via gadolinium-enhanced magnetic resonance imaging (GdMRI). The CSF sICAM-1 (A) and sVCAM-1 (B) levels in patients with NMO with lesions (n=14) and without lesions (n=4) detected via GdMRI. Solid lines indicate the median levels in each group; bars, minimum and maximum values.

Figure 3. Cerebrospinal fluid (CSF) soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1) levels in patients with neuromyelitis optica (NMO) with or without lesions detected via gadolinium-enhanced magnetic resonance imaging (GdMRI). The CSF sICAM-1 (A) and sVCAM-1 (B) levels in patients with NMO with lesions (n=14) and without lesions (n=4) detected via GdMRI. Solid lines indicate the median levels in each group; bars, minimum and maximum values.

The serum antibody against AQP4, which is thickly concentrated in the foot processes of astrocytes at the blood-brain barrier, is a specific marker of NMO. We speculate that blood-brain barrier disruption is necessary for the anti-AQP4 antibody to enter the CNS and that it is involved in the pathogenesis of NMO. However, primary disruption of the blood-brain barrier in NMO may not be caused by the anti-AQP4 antibody itself for the following reasons: astrocytes are outer components of the blood-brain barrier, hence, it is difficult to access them from the peripheral blood; animal models of NMO were not established by the administration of anti-AQP4 antibody in the peripheral circulation; and a patient who possessed the anti-AQP4 antibody years before the onset of NMO was reported. Astrocyes play important roles in forming and reinforcing the blood-brain barrier. An experimental in vitro work has demonstrated the pathogenic role of the anti-AQP4 antibody in causing damage in a blood-brain barrier model. Concretely, anti-AQP4 antibody binding to astrocytes alters AQP4 polarized expression, induces astrocytic death by antibody-dependent cellular cytotoxicity, and increases the permeability of the blood-brain barrier. Immunopathologic studies of NMO indicate that AQP4 immunoreactivity is lost in the perivascular areas and that astrocytic dysfunction occurs in the acute inflammatory lesions of NMO, presumably due to autoimmunity against AQP4. Moreover, remarkable elevations of CSF–glial fibrillary acidic protein levels in the acute phase of NMO suggest that astrocytic damage is involved in the pathogenesis of NMO. These previous observations suggest that once disruption of the blood-brain barrier occurs in NMO, peripheral circulating anti-AQP4 antibody can access astrocytic end-feet, and the influx of humoral or cellular immune components will attack the CNS. However, the cause of primary blood-brain barrier disruption in NMO remains unknown. This process may allow sustained disruption of the blood-brain barrier by astrocytic dysfunction, facilitating the access of anti-AQP4 antibody to astrocytes and further infiltration of the immune components into the CNS. Therefore, patients with NMO may show severe blood-brain barrier disruption and inflammation of the CNS. The elevations of soluble adhesion molecules also may reflect astrocytic damage. However, it has been reported that the elevation of these soluble adhesion molecule levels also reflects brain endothelium activation. Our findings also may reflect that such a condition has occurred in the pathogenesis of NMO.

Some limitations of our study need to be addressed. We could not demonstrate the time when the adhesion molecule levels became elevated because lumbar puncture was rarely performed at the remission phase. Another limitation is that we were able to apply only partial albumin quotient and GdMRI findings because ours was a retrospective study.

In conclusion, our results showed significant elevations of sICAM-1 and sVCAM-1 levels and their correlations with the albumin quotient and with lesions detected via GdMRI, supporting the hypothesis that blood-brain barrier disruption is involved in the pathogenesis of NMO. Measuring the adhesion molecules, especially CSF sICAM-1, may be useful for monitoring blood-brain barrier disruption in NMO. Further research on sICAM-1 and sVCAM-1 in NMO, especially a serial study into their clinical relevance, may provide further insight into their role in the pathogenesis of NMO. Thus, the anti–blood-brain barrier pathway could be a promising target for new NMO pharmacologic treatments.
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