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

Akiyuki Uzawa, MD; Masahiro Mori, MD, PhD; Saeko Masuda, MD; Satoshi Kuwabara, MD, PhD

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). Neuromyelitis 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...
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^(-5), 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>19/25 (76)</td>
<td>14.0 (1.0-29.0)</td>
<td>1/0 (1.0-10.0)</td>
<td>NA</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Positive for anti-aquaporin 4 antibody, No. (%)</td>
<td>3.0 (1.0-29.0)</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 (0.5-0.0)</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>

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.

a Of the total patients, 18 with NMO and 15 with MS underwent GdMRI when serum and cerebrospinal fluid were sampled.

Results

Clinical profiles of patients with NMO, MS, and ONNDs are summarized in the Table. The mean (SD) CSF cell counts (18.4 [27.5]/µL), CSF protein (66.3 [49.4] mg/dL), and other clinical and laboratory findings were compared between the groups.

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).

Clinical Characteristics

Clinical profiles of patients with NMO, MS, and ONNDs are summarized in the Table. The mean (SD) CSF cell counts (18.4 [27.5]/µL), CSF protein (66.3 [49.4] mg/dL), and other clinical and laboratory findings were compared between the groups.
Patients with NMO (patients with NMO than in patients with MS and ONNDs of sICAM-1 and sVCAM-1 were significantly higher in MS and ONNDs (n=25) (B), and CSF protein levels (n=25) (C) in patients with NMO. D-F: Correlations between the CSF sVCAM-1 levels and albumin quotient (D), CSF cell counts (E), and CSF protein levels (F) in patients with NMO.

Figure 2. Correlations of soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1) levels with clinical variables in patients with neuromyelitis optica (NMO). A-C: Correlations between the cerebrospinal fluid (CSF) sICAM-1 levels and albumin quotient (n=15) (A), CSF cell counts (n=25) (B), and CSF protein levels (n=25) (C) in patients with NMO. D-F: Correlations between the CSF sVCAM-1 levels and albumin quotient (D), CSF cell counts (E), and CSF protein levels (F) in patients with NMO.

and albumin quotient × 10² (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 = −.03, P = .87; sVCAM-1: r = −.19, P = .30) or those with MS (sICAM-1: r = .09, P = .72; sVCAM-1: r = .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 [3009.3] 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-
min quotient, and the degree of barrier damage was gradua-
tier disruption in patients with NMO. The integrity of the
adhesion molecules is useful in determining the degree of blood-brain bar-
whether the levels of serum and CSF adhesion molecules
NMO, to our knowledge. In this study, we investigated the
however, no studies have examined these molecules in
MRI results and disease activity in patients with MS,3,14; and
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ated with blood-brain barrier disruption and correlate with
MRI results and disease activity in patients with MS,3,14; however, no studies have examined these molecules in
NMO, to our knowledge. In this study, we investigated the
adhesion molecules sICAM-1 and sVCAM-1 to determine
whether the levels of serum and CSF adhesion molecules
are useful in determining the degree of blood-brain bar-
rier disruption in patients with NMO. The integrity of the
blood-brain barrier was assessed by calculating the albumin
quotient, and the degree of barrier damage was gradu-
ated according to the albumin quotient, as previously de-
scribed. As a result, significant elevations in the levels of sICAM-1 and sVCAM-1 were observed compared with
patients with MS, ONNDs, and HCs, and the correlations
of sICAM-1 levels with the albumin quotient and CSF
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 eleva-
tions of sICAM-1 and sVCAM-1 levels and their correla-
tions with the albumin quotient and with lesions detected
via GdMRI, supporting the hypothesis that blood-brain bar-
rier disruption is involved in the pathogenesis of NMO. Mea-
asuring 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 rele-
vance, 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 phar-
macologic treatments.

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and Masuda. Analysis and interpretation of data: Uzawa,
Mori, and Kuwabara. Drafting of the manuscript: Uzawa.
Critical revision of the manuscript for important intellec-

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.
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REFERENCES