Specificity and Correlation With Disease Activity of Cerebrospinal Fluid Osteopontin Levels in Patients With Multiple Sclerosis

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Background: Despite recent advances in therapy that have improved the overall disease course in multiple sclerosis (MS), the prognosis at the outset remains unpredictable.

Objectives: To investigate whether cerebrospinal fluid (CSF) osteopontin levels correlate with disability or active disease in MS and to determine whether elevated CSF osteopontin levels are only seen in MS.

Design: Cerebrospinal fluid osteopontin was assayed using enzyme-linked immunosorbent assay in duplicate by an observer blinded to the clinical status of the sample. Cerebrospinal fluid samples were not obtained from any patient who had received high-dose corticosteroid therapy in the month before analysis.

Setting: Medical research institute.

Patients: Thirty patients (18 women and 12 men; age range, 24-71 years) with clinically definite MS and 36 patients (22 women and 14 men; age range, 20-71 years) with other neurological diseases (ONDs) or nonneurological illnesses were included in the study.

Main Outcome Measures: Disease activity for patients with MS was based on observations in the year preceding the study, including the number of relapses, the change in disability according to the Expanded Disability Status Scale, and increased T2-weighted or gadolinium-enhancing lesions on brain magnetic resonance imaging.

Results: Higher CSF osteopontin levels were seen in patients with MS having active disease and in patients with ONDs that are actively deteriorating or inflammatory. However, CSF osteopontin levels in patients with MS did not correlate with disability status.

Conclusions: Cerebrospinal fluid osteopontin levels do not correlate with disability in MS but tend to be higher in patients with active disease. Elevated CSF osteopontin levels are not a specific marker for MS, as they are found in patients with ONDs and nonneurological illnesses. In ONDs, the highest CSF osteopontin levels are seen in patients with rapidly progressive neurological dysfunction or widespread inflammation of the central nervous system.

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Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) of unknown cause. At the time of diagnosis, the prognosis is variable because the natural history of the disease ranges from a benign illness to a severe disabling one. Despite recent advances in therapy that have improved the overall outlook of the disease course, the prognosis at the outset remains unpredictable. This is because patient response to treatment is varied and it is difficult prospectively to determine the appropriate level of aggressiveness of therapy. One of the challenges in MS is to identify biomarkers that correlate with future disease severity. Identification of such markers would enable more timely radical treatment approaches in patients who are reliably predicted to have more severe manifestations of disease later.

A possible candidate biomarker that correlates with disease severity is the proinflammatory cytokine osteopontin, produced by immune and nonimmune cells. Among immune cells, it is expressed by T cells, macrophages, and natural killer cells. Nonimmune sources of osteopontin include endothelial cells, epithelial cells, fibroblasts, bone cells, tumor cells, and brain cells (eg, astrocytes and neurons). Chabas et al found increased transcripts of osteopontin in complementary libraries of MS brain plaques. In addition, using microarray analysis of rat brains, they re-
ported increased osteopontin transcripts in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Furthermore, osteopontin-deficient mice were resistant to developing severe EAE. Implications of a possible role of osteopontin in the pathogenesis of MS are intriguing because of its known biological functions. Osteopontin increases interleukin 12 and interferon gamma production and inhibits interleukin 10 production in T cells and macrophages in humans and mice.9,10 These reciprocal effects of cytokine production suggest that osteopontin may be an inducer of proinflammatory responses.8,10 Recent studies11,12 demonstrated elevated plasma osteopontin levels in acute relapsing-remitting MS and in normal-appearing white matter in secondary progressive MS. They have also been detected in breast cancer13 and ovarian cancer14 pathologic tissues and in glioblastomas, in which the tissue osteopontin expression correlated with the histologic grade of the tumor.13

Our objective was 2-fold. The first objective was to determine whether cerebrospinal fluid (CSF) osteopontin levels correlate with disease activity or severity in MS. The second objective was to evaluate whether elevated osteopontin levels are specific to MS.

**METHODS**

**PATIENTS**

Cerebrospinal fluid was obtained by lumbar puncture from 30 patients with clinically definite MS according to criteria by McDonald et al16 (Table 1) and from 36 patients with other neurological diseases (ONDs) or nonneurological illnesses (Table 2). The study was approved by the institutional review board and informed consent was obtained from the participants. There were 18 female and 12 male patients (age range, 24-71 years) with MS. These included 11 with relapsing-remitting disease, 13 with secondary progressive disease, and 6 with primary progressive disease. Eight of 11 patients with relapsing-remitting disease were receiving interferon beta therapy, and 3 were taking glatiramer acetate. Of 13 patients with secondary progressive disease, 11 were receiving interferon beta therapy, 1 was taking glatiramer acetate, and 1 was not receiving treatment. All 6 patients with primary progressive MS were not receiving immunomodulating agents. The control subjects included patients with other inflammatory diseases such as CNS lupus, neurosarcoidosis, and neuroborreliosis, as well as patients with neurodegenerative and neoplastic diseases and with spinal cord injury. There were 22 female and 14 male control subjects (age range, 20-71 years).

**CLINICAL STATUS**

All patients with MS were categorized as having active or inactive disease based on the level of disease activity. Analysis of osteopontin levels, disease activity was scored as 0 for no activity or as 1, 2, or 3 relative to the number of activity criteria present in the patient. Each of the following criteria counted as a score of activity during the 12 months before obtaining CSF samples: 2 relapses or more, a change in the Expanded Disability Status Scale score of at least 0.5, and magnetic resonance (MR) imaging evidence of new disease activity (T2-weighted or gadolinium-enhancing lesions or an increase in lesion number). All patients were assessed at baseline and at 6 and 12 months for the Expanded Disability Status Scale score.

**OSTEOPONTIN ASSAY**

Cell-free CSF was collected by centrifugation at 400g for 15 minutes at 4°C immediately after the lumbar puncture and was aliquoted and stored at −80°C until the osteopontin assay was performed. Quantification of osteopontin levels in CSF samples was performed using a commercially available enzyme-linked immunosorbent assay kit (human osteopontin TiterZyme enzyme immunoassay; Assay Designs, Inc, Ann Arbor, Michigan) according to the manufacturer’s protocol. The assay had a minimum osteopontin concentration detection limit of 5 ng/mL. Cerebrospinal fluid samples were diluted to 1:25, 1:50, 1:100, and 1:200 to determine the linearity and sensitivity of the assay. The assay was most sensitive at a CSF dilution of 1:200, which was used for most samples. The intra-assay and interassay coefficients of variation were 3% to 5% and 1% to 2%, respectively. All assays were performed in duplicate, and the observer (S.A.C.) was blinded to the diagnosis. No CSF sample was obtained from any patient who had received high-dose corticosteroid treatment in the month preceding analysis.

**STATISTICAL ANALYSIS**

Statistical analyses were performed using t test. Two-tailed t test was used to compare the mean (SD) osteopontin concentration. P<.05 was considered statistically significant.
Among all patients with MS, CSF osteopontin levels ranged from 0.6 to 32 µg/mL. There was no correlation between osteopontin levels and the age or sex of the patients. Cerebrospinal fluid osteopontin levels did not correlate with disability in MS as assessed by the Expanded Disability Status Scale score (Table 1).

No statistically significant differences were noted in osteopontin levels among patients with relapsing-remitting, secondary progressive, or primary progressive forms of MS (data not shown). However, the patient with MS having the highest osteopontin level (32 µg/mL) had the relapsing-remitting form of MS with highly active disease at the time of CSF collection, with multiple gadolinium-enhancing lesions on brain MR imaging and a Marburg variant type of presentation.

Patients with ONDs had osteopontin levels ranging from 0 to 18 µg/mL. Again, no age or sex correlations were seen with osteopontin levels. High osteopontin levels (>10 µg/mL) were seen in various conditions, including inflammatory disorders such as systemic lupus erythematosus, primary CNS vasculitis (biopsy based), neuremyelitis optica, and noninflammatory diseases such as gliomatosis cerebri.

Osteopontin levels were not statistically significantly different in patients with MS vs ONDs (Table 2). Osteopontin levels were higher in patients with more severe or active CNS involvement (Table 3). Patients with MS having active disease tended to have higher osteopontin levels than patients who were stable, although this difference was not statistically significant. A linear relationship was seen when disease activity scores were correlated with osteopontin levels. Three patients without disease activity had a mean (SD) osteopontin level of 2.8 (1.4) µg/mL, 10 patients with a disease activity score of 1 had a mean (SD) osteopontin level of 6.4 (4.3) µg/mL, 16 patients with a disease activity score of 2 had a mean (SD) osteopontin level of 7.4 (4.8) µg/mL, and the only patient with a disease activity score of 3 had an osteopontin level of 32 µg/mL.

In patients with ONDs, osteopontin levels were statistically significantly higher in patients with active disease. The diagnoses of 7 patients with ONDs who had active disease were as follows: Leigh disease (patient deceased), gliomatosis cerebri (patient deceased), primary CNS vasculitis, neuremyelitis optica, active CNS lupus (myelitis), neuromyelitis optica, and cerebellar degeneration.

Plasma osteopontin levels are suggested to be a marker of disease activity in MS, and increased expression in the brain has been associated with more severe manifestations of disease.15 If confirmed, these observations are important because osteopontin level may serve as a biomarker of severe manifestations of disease, with therapeutic and prognostic implications. Our CSF-based study was designed to answer the following 2 questions: do CSF osteopontin levels correlate with disease activity in MS and is an elevated osteopontin level a specific marker for MS?

Our results show unequivocally that elevated osteopontin levels in CSF are not MS specific, as we found no statistically significant difference in osteopontin levels between patients with MS and control subjects. The correlation of osteopontin level with disease activity is more difficult to compare because disease activity was more readily defined in the patients having MS than in our control group having disparate diseases. Despite the arbitrary disease activity classification, osteopontin levels in CSF seem to correlate with rapidly progressive and widespread CNS disease.

In patients with MS, CSF osteopontin levels did not correlate with disability (Table 1), but a definite trend toward higher CSF osteopontin levels in patients with active disease was seen. If osteopontin level is related to disease activity in MS, it could be rationally explained on the basis of the known actions of osteopontin as a mediator of leukocyte inflammatory responses.9,10

The most striking feature of our data was that osteopontin levels were elevated in patients with severe neurological disease. Healthy control subjects and patients who had stable neurological diseases such as cervical spondylosis or spinal cord injury had statistically significantly lower CSF osteopontin levels compared with patients who had active inflammatory diseases such as CNS vasculitis, gliomatosis, and active CNS lupus (Table 3). Also, the patient with MS having the highest CSF osteopontin level had the most rapid demyelination and the greatest number of gadolinium-enhancing lesions. Together, these results suggest that CSF osteopontin level correlates best with CNS inflammation or with blood-brain barrier breakdown and is not disease specific.

Further support for the role of osteopontin in inflammatory diseases of the brain has been provided by work on EAE in mice.16 In that study, osteopontin induced relapses and disease progression in EAE through enhanced survival of activated T cells. At a molecular level, the survival of the T cells was mediated by enhanced inhibition and activation of different transcription factors in activated T cells and by altered expression of proapoptotic proteins. These observations have not been extended to MS but suggest plausible mechanisms for the elevation of CSF osteopontin levels in active disease.

Other investigators have described elevated osteopontin plasma levels in patients with active MS.11,17 This is supported by the data in our present CSF study. Early findings suggested that elevated osteopontin levels correlated

### Table 3. Cerebrospinal Fluid Osteopontin Levels in Patients With Stable and Active Multiple Sclerosis and Other Neurological Diseases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Disease Activity</th>
<th>No. of Patients</th>
<th>Osteopontin Level, Mean (SD), µg/mL</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple sclerosis</td>
<td>Stable</td>
<td>13</td>
<td>5.6 (4.1)</td>
<td>.18</td>
</tr>
<tr>
<td></td>
<td>Active</td>
<td>17</td>
<td>8.8 (7.6)</td>
<td></td>
</tr>
<tr>
<td>Other neurological disease</td>
<td>Stable</td>
<td>29</td>
<td>4.3 (2.7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Active</td>
<td>7</td>
<td>11.8 (6.0)</td>
<td></td>
</tr>
</tbody>
</table>
with disease progression. However, more recent work with plasma samples, as well as our CSF findings, shows a lack of correlation between disease progression and osteopontin levels. A study correlating plasma and CSF osteopontin levels among the same patients with MS and control subjects perhaps would help further clarify the results.

Although we did not find a specific correlation between disease progression and CSF osteopontin levels in MS, our data suggest that increased disease activity is associated with higher intrathecal osteopontin levels. Therefore, it is possible that CSF osteopontin level could be used as a biomarker of therapeutic efficacy for a drug such as natalizumab, which is believed to act by stabilizing the blood-brain barrier. In treatment-responsive patients, high pretreatment levels of CSF osteopontin would be expected to decrease, resulting in low posttreatment levels following a period of therapy. Continued disease activity with unaltered CSF osteopontin levels in unresponsive patients may indicate antibody development to the medication and provide a rational basis for changing therapy.

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REFERENCES


