Agarose Electrophoresis and Immunonephelometric Quantitation of Cerebrospinal Fluid Immunoglobulins: Criteria for Application in the Diagnosis of Neurologic Disease

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The cerebrospinal fluid (CSF) IgG index, the CSF to serum albumin ratio, and electrophoresis on agarose gel of CSF and serum were evaluated retrospectively for their usefulness in the differential diagnosis of multiple sclerosis (MS). Standardized procedures were adopted for grading the intensity of oligoclonal banding and for the certainty of diagnosis of MS. One hundred and forty-nine patients were studied, including 23 with definite multiple sclerosis (MS), 12 with probable MS, 20 with possible MS, 20 with inflammatory neurologic disease, 65 with noninflammatory neurologic disease, and nine with no neurologic disease. The CSF IgG index and the CSF to serum albumin ratio were calculated from nephelometric measurements of serum and CSF IgG and albumin. The intensity of oligoclonal banding was graded relative to the density of the prealbumin band.

Eighty-eight per cent of cases of definite MS had distinct oligoclonal bands, and an equal number had an elevated IgG index. These tests were not specific for MS, however, since 50% of cases of inflammatory neurologic disease and 5% of those with noninflammatory neurologic disease had an elevated IgG index. Similarly, 48% of cases with inflammatory disease and 25% with noninflammatory disease had oligoclonal bands. However, only patients with definite MS (21%) or possible MS (4%) had prominent oligoclonal bands whose density was greater than or equal to that of prealbumin, together with a CSF IgG index greater than 1.50. This combination of findings therefore may enhance the level of suspicion of MS. By contrast, an isolated increase in the CSF to serum albumin ratio may suggest a diagnosis other than MS. (Key words: Multiple sclerosis; IgG index; Oligoclonal bands; Agarose gel electrophoresis) Am J Clin Pathol 1984; 81: 575-580

MULTIPLE SCLEROSIS (MS) is the most common chronic neurologic disease in young adults. The diagnosis of MS, particularly in the early stages, can be difficult because of the diversity of potential symptoms and because of its resemblance to other diseases. Thus, several classification schemes exist for the diagnosis of MS and most include the categories of "possible" and/or "probable MS" in addition to "definite MS." In recent years, the clinician has been aided in the diagnosis of MS by clinical laboratory tests as well as by other studies, including evoked potentials, computerized tomography (CT), and nuclear magnetic resonance scanning. Even with the incorporation of these tests into the most recent criteria proposed for the diagnosis of MS, the category of "probable MS" could not be eliminated.

Increased synthesis of IgG, including oligoclonal immunoglobulins of restricted heterogeneity, has been demonstrated within the central nervous system (CNS) in patients with MS. Although the role of these antibodies in the pathogenesis of MS is uncertain, evaluation of their presence in the CNS has added to the diagnostic workup. Electrophoresis of CSF and serum is used to look for oligoclonal immunoglobulins. Measurements of serum and CSF IgG and albumin often are combined in a ratio, such as the CSF IgG index or the CSF IgG synthesis rate. By using albumin, which is synthesized by the liver, as a marker of blood-brain barrier breakdown, these ratios compensate for passive leakage of IgG into the CNS and assess localized IgG synthesis.

Although either oligoclonal banding or immunoglobulin synthesis ratios are positive in 80-90% of patients with definite MS, these results are not specific for MS. In fact, oligoclonal bands and increased IgG synthesis in the central nervous system are seen in patients with infectious neurologic disease. The purpose of this study was to determine quantitative criteria for the level of the CSF IgG index and/or the intensity or number of oligoclonal bands that might better distinguish patients with MS from those with other neurologic diseases.

Materials and Methods

We retrospectively reviewed 157 consecutive studies of CSF and serum from patients seen at the Emory University Affiliated Hospitals or Clinic over a two-year period. One of us (GSP) also reviewed the patients' charts and classified the patients based on standardized clinical criteria. When individual patients were studied more than once, the number of studies in that category is in-
Dedicated in parentheses. We studied 23 (24) patients with definite MS, 12 (14) patients with probable MS, and 20 (23) patients with possible MS. Seventeen of the patients with definite MS had a relapsing–remitting course, while six had a chronic progressive course.

We also studied 20 (21) patients with inflammatory neurologic diseases, including those of infectious and immunologic etiology, 65 (66) patients with noninflammatory neurologic diseases, and nine patients with nonneurologic diseases. Patients with infectious diseases included those with viral encephalomyelitis, bacterial intracerebral abscess, neuroretinitis, neurosyphilis, toxoplasmosis, and infectious vasculitis. The category of immunologic disease included patients with CNS sarcoidosis, Guillain–Barre syndrome, mixed connective tissue disease with neuropathy, rheumatoid arthritis with neuropathy, Sjogren’s syndrome with rheumatoid arthritis and neuropathy, and systemic vasculitis. Patients with noninflammatory neurologic diseases were subdivided into degenerative (dementia of the Alzheimer type, dorsal column myelopathy, spinocerebellar ataxia, and amyotrophic lateral sclerosis), malformative (Chiari I malformation, arachnoid cyst, empty sella, and vascular malformation), metabolic (diabetic neuropathy, subacute combined degeneration, Niemann–Pick disease, anoxic encephalopathy, and hepatic encephalopathy), neoplastic (meningioma, astrocytoma, paraneoplastic syndromes, and lymphomatous meningitis), and miscellaneous (peripheral neuropathy, headache, pseudotumor cerebri, old spinal trauma, Jakob–Creutzfeldt disease, cerebral infarction, abnormal gait of unknown etiology, Brown–Sequard syndrome, hydrocephalus, myopathy, radiation-induced myelopathy, and sacral root compression) categories.

All of the tests had been performed in the Clinical Immunology Laboratory of Emory University Hospital. CSF and serum were centrifuged to remove cells and aliquots of the supernatant were stored either at 4°C for no more than 72 hours for nephelometry or at −70°C for electrophoretic studies. IgG and albumin were measured immunochemically using the Beckman Immunochemistry System (ICS) Nephelometer® with Beckman anti-IgG and albumin antisera, calibrators, and control standards as reagents (Beckman Instruments, Inc., Fullerton, CA). The CSF IgG index was computed using the following formula:

$$\text{IgG Index} = \frac{(\text{CSF IgG/serum IgG})}{(\text{CSF albumin/serum albumin})}.$$ 

Values greater than 0.70 were 3 SD above the mean of normal samples tested in our laboratory and were considered abnormal. The ratio of CSF albumin to serum albumin was computed to assess the integrity of the blood–brain barrier using established criteria.32

For agarose gel electrophoresis, CSF was concentrated to 2 g/L by ultrafiltration using a Minicon® CS-15 concentrator (Amicon Corp., Lexington, MA) and serum was diluted 1:6 in normal saline. Ten microliters of each was applied for electrophoresis. During the first 19 months of the study, the Panagel Electrophoresis Reagent Set® was used with the Panagel Migration Unit, Slides, and Electrode Buffer® (Worthington Diagnostics, Freehold, NJ), pH 8.6. During the last five months, the 1% agarose gel plates (SeaKem® agarose, Marine Colloids, FMC Corp., Rockland, ME), were prepared in our laboratory and the LKB Multiphor Electrophoresis System® (LKB Instruments, Inc., Stockholm, Sweden) was used. Both systems gave comparable results. Electrophoresis was performed at 20 V/cm for 45 minutes and gels were fixed in picric and acetic acid (Worthington Diagnostics) and stained with 0.5% Coomassie blue.

All gels were evaluated for the presence of oligoclonal immunoglobulin bands in CSF. These were defined as one or more bands in the gamma region that were not present in serum and that did not include beta or gamma trace protein.17 The intensity of the densest band based on visual inspection of the membrane determined the classification of the pattern as follows: prominent bands were as dense or denser than the prealbumin band, moderate bands were distinct bands that were less dense than prealbumin, faint bands were indistinct bands on whose presence two investigators did not agree, and absent bands were either not present in the CSF or were identical to those in serum (Fig. 1). Prealbumin was chosen as an internal standard, since it is not synthesized in the CNS and its density on electrophoresis may overlap with that of the most prominent oligoclonal bands. The use of standards other than albumin has been discussed.9
CSF ANALYSIS IN NEUROLOGIC DISEASE

Table 1. Patterns of CSF Response in Patients With Neurologic Disease (Per Cent of Patients)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
<th>Oligoclonal Bands*</th>
<th>CSF IgG Index</th>
<th>Incr Perm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absent</td>
<td>Faint</td>
<td>Moderate</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.70</td>
</tr>
<tr>
<td>Definite</td>
<td>24</td>
<td>8</td>
<td>4</td>
<td>58</td>
</tr>
<tr>
<td>Probable</td>
<td>14</td>
<td>7</td>
<td>14</td>
<td>64</td>
</tr>
<tr>
<td>Possible</td>
<td>23</td>
<td>35</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>Inflammatory diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunologic disease</td>
<td>9</td>
<td>78</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Infectious disease†</td>
<td>12</td>
<td>25</td>
<td>8</td>
<td>58</td>
</tr>
<tr>
<td>Noninflammatory diseases‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degenerative disease‡</td>
<td>12</td>
<td>82</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Malformations§</td>
<td>6</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Metabolic diseases§</td>
<td>8</td>
<td>50</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Neoplastic diseases‡</td>
<td>8</td>
<td>43</td>
<td>14</td>
<td>43</td>
</tr>
<tr>
<td>Miscellaneous diseases‡</td>
<td>32</td>
<td>61</td>
<td>10</td>
<td>29</td>
</tr>
</tbody>
</table>

* Patterns of oligoclonal bands as defined in "Materials and Methods.”
† Nephelometry not done in one case.
‡ Electrophoresis not done in two cases.

Results

An elevated CSF IgG index was found in patients with various neurologic diseases. Eighty-eight per cent of the patients with definite MS, 64% with probable MS, 17% with possible MS, and 50% with inflammatory neurologic disease had a CSF IgG index greater than 0.70. Only 5% with noninflammatory neurologic disease had a CSF IgG index greater than 0.70, and none of the patients without neurologic disease had this abnormality (Table 1). The patients with definite MS tended to have the highest CSF IgG index. In fact, an IgG index greater than 1.50 frequently was seen in patients with definite or probable MS but was uncommon in the other disease classes (Fig. 2, Table 1).

Among the patients with definite MS, the level of the CSF IgG index did not correlate with the duration of disease (r = —0.15, P > 0.1, Spearman rank order correlation) or the age of onset of MS (r = —0.13, P > 0.1, Spearman rank order correlation). Further, the level of the CSF IgG index did not relate to the presence of periventricular plaques on CT scans. Two of the patients with confirmed periventricular plaques had a CSF IgG index less than 0.7, five had an index between 0.7 and 1.5, and only one had an index greater than 1.5.

The initial evaluation of oligoclonal banding patterns revealed that faint bands are not clinically significant and commonly are seen in the CSF when using sensitive agarose gel electrophoresis. Therefore, only definite bands that were categorized as moderate or prominent were considered positive. Using these criteria, 88% of cases of definite MS had oligoclonal bands in the CSF, while 79% of probable MS, 52% of possible MS, 48% of inflammatory neurologic disease, 25% of noninflammatory neurologic disease, and none with no neurologic disease had these bands. The patients with definite or possible MS more often had prominent bands, whereas these were uncommon in the other disease classes (Fig. 2, Table 1).

Among patients with definite MS, those with prominent bands did not differ from those with moderate bands with respect to the duration of disease (U = 57, P > 0.1, Mann–Whitney U Test) or the age of onset of disease (U = 23, P = 0.1, Mann–Whitney U Test). Of the seven patients who had multiple tests, there was no change in the intensity of the bands in three, a decrease from moderate to absent bands in two, and an increase from moderate to prominent bands in two. The intensity of the bands did not appear to reflect the proximity of disease to the ventricles, since six patients with periventricular plaques had moderate bands and only two had prominent bands.

The number of oligoclonal bands did not appear to have much clinical significance. It did not correlate with the duration (r = —0.16, P > 0.10, Spearman rank order correlation) or age of onset of disease (r = —0.05, P > 0.1, Spearman rank order correlation) in patients with definite MS. However, patients with noninflammatory neurologic disease who had oligoclonal bands tended to have fewer bands than did patients with definite MS (z = —2.18, P = 0.03, Mann–Whitney U Test). Patients with inflammatory neurologic disease who had oligoclonal bands, however, had numbers of bands comparable to patients with definite MS (z = —0.49, P = 0.62, Mann–Whitney U Test).

An elevated CSF IgG index together with oligoclonal banding in the CSF was highly suggestive of MS in our patient population. This combination had a specificity of 99% and a sensitivity of 79%, if we omitted cases of inflammatory neurologic disease, which generally are differentiated from MS by other laboratory and clinical criteria. More interestingly, a pattern of both prominent oligoclonal bands and a CSF IgG index greater than 1.5
was seen only in five patients with definite MS and one patient with possible MS (Fig. 2). Two of these patients with definite MS had chronic progressive disease, while three had disease of the remitting-relapsing type. One patient with remitting-relapsing MS had entered a progressive phase, one was in remission, and one was in relapse.

Blood-brain barrier permeability commonly was increased in all of the classes of neurologic disease (Table 1). However, an isolated defect in permeability without oligoclonal bands or an elevated IgG index was seen in very few patients with MS (4% of definite, 7% of probable, 9% of possible) but in a relatively larger proportion of patients with inflammatory (16%) or noninflammatory (21%) neurologic disease. Thus, an isolated defect in blood–brain barrier permeability might suggest a disorder other than MS, as previously described.5

Single bands of the same immunoglobulin type were

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Fig. 2. The CSF IgG index and pattern of oligoclonal banding for each patient studied. Note that prominent oligoclonal bands together with a CSF IgG index greater than 1.5 are seen only in patients with definite or possible multiple sclerosis (MS). Prm = prominent; Mod = moderate; Fnt = faint; Abs = absent; • = definite MS; O = probable MS; O = possible MS.
present in the CSF and serum in two patients having paraproteins, one with cerebellar degeneration and the other with peripheral neuropathy. Multiple identical bands were seen in CSF and serum in two patients with peripheral neuropathy, one with mixed connective tissue disease and the other with rheumatoid arthritis. These may represent serum-derived immune complexes, since such bands in serum frequently correspond to immune complexes.15

**Discussion**

Clinical laboratory testing, including the CSF IgG index and assessment of oligoclonal banding on electrophoresis of CSF and serum, has become an important part of the diagnostic evaluation of patients with neurologic disease. However, few attempts have been made to grade the intensity of oligoclonal bands, and no guidelines for the interpretation of weak versus distinct bands have been published. The purpose of this study was to determine if quantitation of the degree of abnormality on these tests might add to their significance in the differential diagnosis of MS.

The percentages of our patients with definite MS who had oligoclonal banding or an elevated IgG index are comparable to those previously reported.1,2,3,7,10-14,16,18,20,25,31 Based on our data, both tests had a sensitivity of 88%, but the specificity for the CSF IgG index was 95%, while that of oligoclonal banding was only 79%. Oligoclonal bands have been reported in some patients with each of the disorders in which we observed them.4,6,10,16,23,31

The tests most suggestive of MS were a markedly elevated CSF IgG index and prominent oligoclonal bands with a density greater than the prealbumin band. Although these criteria are too stringent to be used as cutoff points for a positive test, the presence of a markedly increased IgG index and prominent oligoclonal bands may increase the level of suspicion for MS. One patient in our series with both tests strongly positive had "possible MS." Continued evaluation of this patient, as well as other patients with similar findings, is necessary to see if this pattern will predict progression of possible MS to definite MS.

The level of the IgG index, the prominence of the oligoclonal bands, and the number of bands did not correlate with the age of onset or duration of illness in patients with MS, in agreement with other reports.3,8,16,28 Several investigators have shown a relationship between the intensity of the immune response and the severity of disease in patients with MS.2,6,16,24,30 The severity of disease was not evaluated in detail in this study because of the difficulty in doing so based on medical records. However, patients with the most highly abnormal tests did not have necessarily the most widespread disease, whereas many with mildly abnormal or normal tests had less severe disease.

Oligoclonal immunoglobulin bands frequently were seen in patients with infectious neurologic diseases. By contrast, they were uncommon in those with other neurologic diseases, including degenerative diseases. We have observed instances in which the presence of oligoclonal bands has directed the clinician toward further workup for infection, such as a brain biopsy or specific serologic studies. Thus, CSF and serum electrophoresis should not be restricted to the evaluation of patients with MS, since the results also may be used to support a diagnosis of central nervous system infection.

**References**

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