Relative Value of Three Laboratory Methods in the Diagnosis of Multiple Sclerosis

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We compared three methods of analysis for IgG in cerebrospinal fluid, using samples from 158 patients with clinically suspected multiple sclerosis and from 200 neurological controls. The tests were: search for oligoclonal bands, calculation of rate of synthesis of IgG in the cerebrospinal fluid, and determination of the IgG/albunin ratio. Paired cerebrospinal fluid and serum samples were collected and their IgG and albumin concentrations measured. Oligoclonal bands were detected by electrophoresis on agarose. Positive results were obtained in 94, 75, and 67% of patients with probable or definite multiple sclerosis by the three respective methods. In contrast, for patients for whom the clinical diagnosis of multiple sclerosis was considered possible, positive results were obtained in 10, 43, and 13%, respectively. Evidently, detection of oligoclonal bands remains the best single test for the presence of abnormal IgG in suspected multiple sclerosis patients. A combination of the first two tests is most sensitive for both probable and definite multiple sclerosis (97%) and possible multiple sclerosis (50%). Some infectious or immunologic disorders can also produce these IgG abnormalities, but they can usually be distinguished from multiple sclerosis by other clinical and laboratory data.

In 1940 Yahr et al., using an immunochromatographic method, measured the ratio of IgG to total protein in the CSF of MS patients and found it to be above normal in two-thirds of the cases (1).2 Tourtellotte extended this observation and simplified the test by using an electrophoresis technique. He also showed that the IgG/albunin ratio was essentially identical with the ratio of IgG to total protein (2). Numerous studies confirm that the former ratio is increased in 50–80% of definite or suspected MS patients (3). Frick and Scheid-Seydel used 32P-labeled IgG to confirm the hypothesis that IgG can be synthesized within the central nervous system (4). Tourtellotte derived a formula for the quantitation of this CNS-derived IgG (de novo synthesis) (5). The formula takes into consideration the IgG that diffuses across the blood-brain barrier in both normal and disease states.

Aagarose electrophoresis of CSF from MS patients reveals discrete bands in the gamma-globulin zone, which is not present in normal CSF (6–8). These are called "oligoclonal bands" (7). Laterre identified these bands as IgG and described the protein as being IgG of "restricted heterogeneity" (3). Electrophoresis of CSF on agarose gel has proved to be a very successful method for the laboratory confirmation of MS in cases where the clinical diagnosis is strongly suspected. This paper presents our experience with the laboratory confirma-

tion of demyelinating disease in patients for whom the diagnosis of MS varies from definite to possible.

Materials and Methods

Clinical Material

All probable or definite MS patients included in this study fulfilled the clinical criteria as outlined by Schumacher et al. (9). The patient’s diagnosis was considered possible MS if symptoms were early, entirely subjective, or if the formal neurological examination by two or more clinical neurologists revealed no or equivocal deficit. The neurological controls were patients seen in consultation by the neurology service, with a clinical diagnosis other than MS. Patients with other neurological diseases known to produce oligoclonal bands are listed separately.

Laboratory Methods

Oligoclonal bands. For agarose electrophoresis we used the “Panagel” migration unit (Warington Diagnostics, Freehold, NJ 07728) and pre-formed agarose slides. CSF samples were concentrated 80-fold (from 2.5 mL to approximately 30 µL) in CS-15 cells (Amicon Corp., Danvers, MA 01923) or until the IgG concentration in the concentrated CSF was 80 to 100 mg/L. A 10-µL aliquot of this concentrated CSF was applied to the agarose slide with a P-20 (2-20 µL) Pipettman (Gilson Medical Electronics, Inc., Middleton, WI 53562). The slides were stained with Amido Black to make the separated proteins visible. A sample was considered positive if there were two or more discrete bands present in the gamma-globulin zone that were not found in the corresponding serum sample. A single band may represent (a) an artifact, (b) the non-IgG gamma trace protein, or (c) diffusion of monoclonal IgG from serum, and hence was not interpreted as a positive test.

IgG/albunin ratio. The quantity of IgG and albumin in the CSF samples was determined by electrophoresis on agar–agarose gels (10). Samples containing abnormally high concentrations of IgG or albumin were diluted with PBS and run again until values obtained fell within the limits of the assay standards. The normal range for the IgG/albunin ratio is 9–25%. The ratio is considered positive in our laboratory when it exceeds 25%.

CNS synthesis of IgG. The rate of de novo synthesis was calculated according to Tourtellotte's formula as follows:

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\text{CNS IgG synthesis (mg/day)} = \left( \frac{\text{IgG}_{\text{CSF}} - \text{IgG}_{\text{S}}}{369} \right) \left( \frac{\text{Alb}_{\text{CSF}} - \text{Alb}_{\text{S}}}{230} \right) \left( \frac{\text{IgG}_{\text{S}}}{\text{Alb}_{\text{S}}} \right) \left( 0.43 \right) \times 5
\]

IgGCSF and IgGS are the respective IgG concentrations in CSF and serum, in milligrams per deciliter, and AlbCSF and AlbS are the albumin concentrations in CSF and serum, in milligrams per deciliter. Serum IgG and albumin were determined by the same method as that used for the CSF samples except that the serum samples were diluted 200-fold with PBS before assay. The normal range for CNS synthesis is -14.0 to +5.6 mg IgG synthesized per day. The synthesis rate is considered abnormal when it exceeds +6 mg/day.

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2 Nonstandard abbreviations: PBS, phosphate-buffered isotonic saline; CSF, cerebrospinal fluid; OCB, oligoclonal bands; CNS, central nervous system; MS, multiple sclerosis; and IgG, immunoglobulin G.

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Results

The concentrations of IgG and albumin were measured in the CSF and serum of the patients studied. Given these data, the IgG/albumin ratio and the amount of IgG synthesized within the CNS can be calculated.

A frequently asked question in our laboratory is: How often is only one of the tests positive? In an attempt to answer this question we graphed the results from three groups of patients (Figure 1). The axes represent the point of intersection between normal and abnormal for the IgG/albumin ratio (y-axis) and IgG synthesis (x-axis). By examining the number of points in each quadrant one can appreciate more readily the correlation between the tests.

Nearly all of the neurological controls (Figure 1A) have normal values for all three assays. Among the patients categorized clinically as having possible MS (Figure 1B), IgG synthesis was abnormally high in 14 patients, the IgG/albumin ratio was increased in five cases (three of whom had increased synthesis), and two patients exhibited OCB as the only abnormality. We believe that it will be important to follow the cases of these patients to determine whether or not they go on to develop clinically definite MS and OCB. Patients with clinically probable or definite MS are shown in Figure 1C. All but four of these patients had positive determinations on one or more of the three tests. The laboratory confirmed the clinical diagnosis by demonstrating OCB as the only abnormality in 20 cases.

By inspection one can see that, in any of the categories of patients studied, very few have an above-normal ratio in the absence of positive synthesis (right-lower quadrants of the Figure sections). The exception to this rule is found in patients with a partial reversal of their serum albumin/globulin ratio, such as occurs in patients with hypoalbuminemia regardless of cause. When this ratio decreases below 1.5, the CSF proteins reflect this change and the IgG/albumin ratio becomes positive, a point that has been emphasized previously by us and others.

Table 1 summarizes our studies of multiple sclerosis patients and neurological controls. The presence of qualitatively distinct (oligoclonal) IgG is the commonest single indicator of abnormal IgG production in the CSF. Stated differently, it one could run only a single test on CSF and had at least 25 mL available, this assay would yield the highest positivity rate in patients suspected of having MS. However, the calculation of synthesis of IgG in the CNS adds more information when one is studying patients in whom the clinical diagnosis is possible MS. As the second column in Table 1 indicates, 43% of the patients with possible MS had abnormal values for CNS synthesis of IgG, as compared with 10% with oligoclonal bands.

Table 1. Comparison of Three Methods for the Laboratory Confirmation of the Diagnosis of Suspected Multiple Sclerosis

<table>
<thead>
<tr>
<th>Laboratory test</th>
<th>Diagnostic category</th>
<th>Definite or probable MS</th>
<th>Possible MS</th>
<th>Neurological controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligoclonal bands</td>
<td>94% (110/118)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10% (4/40)</td>
<td>0% (0/200)</td>
<td></td>
</tr>
<tr>
<td>IgG synthesis</td>
<td>75% (89/118)</td>
<td>43% (14/40)</td>
<td>1.5% (3/200)</td>
<td></td>
</tr>
<tr>
<td>IgG/Alb ratio in CNS</td>
<td>67% (79/118)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13% (5/40)</td>
<td>0% (0/200)</td>
<td></td>
</tr>
</tbody>
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<sup>a</sup> Numbers in parentheses represent number positive over number assayed.

<sup>b</sup> Among the 33% of patients with normal ratios, 54% had OCB and 38% had increased rates of IgG synthesis.

![Fig. 1. IgG synthesis rate as a function of the IgG/albumin ratio in percent](image)

A, neurological control group; B, possible MS patients; and C, probable or definite MS. The intersection of the x and y axes in all three sections represent the cross-over point between normal and abnormal for IgG synthesis and the IgG/Alb ratio, respectively. Data identified with * are patients with CSF OCB and those with # are patients without CSF OCB.

Noteworthy is the fact that when one knows the results of the OCB and CNS synthesis tests, calculation of the IgG to albumin ratio adds nothing per se to the diagnostic sensitivity.
Table 2. Other Diseases with Positive Laboratory Findings

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>Test</th>
<th>IgG synthesis</th>
<th>IgG/Albumin ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis</td>
<td>OCB</td>
<td>3/5 (b)</td>
<td>2/5</td>
</tr>
<tr>
<td>Guillain–Barré</td>
<td>IgG synthesis</td>
<td>5/12</td>
<td>11/14</td>
</tr>
<tr>
<td>Encephalitis or meningitis</td>
<td>IgG synthesis</td>
<td>8/14</td>
<td>13/15</td>
</tr>
<tr>
<td>SLE</td>
<td>IgG synthesis</td>
<td>2/11</td>
<td>3/11</td>
</tr>
<tr>
<td>CJD</td>
<td>IgG synthesis</td>
<td>1/1</td>
<td>N.D.</td>
</tr>
<tr>
<td>Recurrent FUO</td>
<td>IgG synthesis</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Sarcoid</td>
<td>IgG synthesis</td>
<td>1/1</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

Abbreviations: SLE, systemic lupus erythematosus; CJD, Creutzfeldt–Jakob disease; SSPE, subacute sclerosing panencephalitis; FUO, fever of unknown origin; and N.D., not determined.  

Another indication of the relative insensitivity of the IgG/albumin ratio is that abnormal rates of IgG synthesis were found in 38% of MS patients with normal IgG/albumin ratios. Over half of these same MS patients (54%) had demonstrable oligoclonal bands; presumably, the appearance of oligoclonal IgG in these patients reflects an abnormality associated with IgG synthesized by CNS lymphocytes and not by circulating lymphocytes.

Table 2 lists other diseases in which we have observed persistently abnormal results in the qualitative and quantitative tests. The diseases listed all have an infectious or immune etiology and therefore one might reasonably expect to find IgG abnormalities. It is worth noting that the laboratory test of choice in Guillain–Barré syndrome is the calculation of the rate of IgG synthesis. A possible explanation for this is that the alteration in the blood–brain barrier in this disease allows serum proteins to enter the CSF, which tends to lower the IgG/albumin ratio in the CSF in spite of ongoing production of IgG in the CNS. Abnormal (oligoclonal) IgG produced in the CSF is masked by the influx of polyclonal IgG from the serum.

Multiple sclerosis continues to be the disease most often responsible for the presence of OCB in the CSF. Nevertheless, in several less-frequent diseases IgG abnormalities may be present (Table 2). Other authors have reported similar findings (11, 12). Clinicians and laboratory personnel need to be alert to the fact that a positive value for any of these tests is important, but sometimes can be explained by the presence of another, possibly treatable, disease.

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References