Simultaneous Determination of Cerebrospinal Fluid Oligoclonal Bands and the "γ-Protein Index" by Agarose Electrophoresis and Densitometry

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Oligoclonal bands were identified in electropherograms of cerebrospinal fluid, and the "γ-protein index" was concurrently calculated from the same strip. For the index, an upper limit of normal of 0.66 was established. We compared results with the clinical diagnosis in 69 patients with multiple sclerosis and 48 control patients with other diseases. Sensitivity, specificity, and positive predictive values of 73%, 96%, and 96%, respectively, were obtained from the index. An abnormal index and the presence of oligoclonal bands combined increased the positive predictive value to 100%. This approach may allow adequate qualitative and quantitative assessment of gamma-globulin abnormalities in cerebrospinal fluid after a single laboratory procedure.

Additional Keyphrases: multiple sclerosis • gamma-globulin abnormalities

In multiple sclerosis (MS) a key finding is increased synthesis of gamma-globulin in the central nervous system, leading to an increase in gamma-globulin in cerebrospinal fluid (CSF). In 1948, Kabat et al. (1) applied a quantitative immunological protein assay for albumin and globulin and produced further evidence that the amount of gamma-globulin in the CSF of patients with neurosyphilis and multiple sclerosis is definitely increased.

More recently, quantification of the IgG and albumin in CSF and serum by immunochemical methods (5–4) has been used to calculate an additional disease-related variable, the IgG index, which is expressed as (5):

\[
\text{(CSF IgG/CSF albumin)/(serum IgG/serum albumin)}
\]

In other often-used calculations, the data on CSF and serum albumin and IgG are used to describe the rate of de novo synthesis of IgG (6), the percentage of IgG in CSF total protein (7), or the IgG/albumin ratio in CSF (8). Each of these calculated values has been used to demonstrate increased synthesis of IgG within the central nervous system. IgG and albumin have been quantified by such immunochemical methods as "rocket" immunoelectrophoresis (2), radial immunodiffusion (3), and nephelometric immunoassay (4).

The presence of multiple bands of restricted electrophoretic mobility in the γ-globulin area is supportive, but nonspecific, evidence for the diagnosis of MS (9–11). Earlier reports suggest that occasional patients with MS may have an abnormal IgG synthesis rate even though their gel-electrophoresis patterns are normal (12) or may have abnormal agarose gel patterns but normal immunoassay results (13). The implication of this is that both electrophoresis and specific immunoassays probably should be used in individual cases. Protein electrophoresis of CSF and serum provide both quantitative and qualitative information about major protein components. Albumin and gamma-globulin are easily quantified by densitometric scanning of stained agarose gels, and "oligoclonal bands" can be readily made visible. Densitometric quantification of albumin and gamma-globulin provides data that can be used to derive the "γ-protein index," with the same formula as for the calculation of the IgG index. We therefore investigated the feasibility of obtaining both qualitative and quantitative information from this single laboratory test, and report our findings here.

Materials and Methods

Specimens. We analyzed 117 consecutively received pairs of CSF and serum samples submitted for oligoclonal band identification. Values for protein in CSF and serum were determined by a biuret method (14). CSF was concentrated in an Amicon A25 concentrator (Amicon Corp., Danvers, MA 01923).

The patients were classified according to diagnosis (Table 1), without prior knowledge of the laboratory findings. Sixty-nine patients met the clinical criteria (15) for definite MS. Five patients were thought to have possible MS, and 43 patients (used as control subjects) had other diagnoses. The miscellaneous diagnoses for the control subjects included: acoustic neuroma, acute renal failure, atypical facial pain, basilar artery insufficiency, cervical disc disease, Chiari-type I malformation, idiopathic chorea, central nervous system lupus, diabetic polyneuropathy, idiopathic dystonia, encephalopathy/hypoglycemia, fever of unknown origin, headache, hysteria, low back pain, motor neuron disease, normal pressure hydrocephalus, papillitis, paroxysmal vertigo, peripheral neuropathy (etiology unknown), pituitary tumor, post-infectious transverse myelitis, brain tumor, pseudo-dementia, segmental myoclonus, and spinocerebellar degeneration. The use of patients rather than normal subjects as controls is based on the belief that the clinical utility of any diagnostic test can best be assessed by using this test in patients whose diseases are likely to be confused with the disease in question.

Electrophoresis reagents. We used agarose-M (LKB Instruments Inc., Rockville, MD 20852) for electrophoresis.

Table 1. Patients Included in the Study

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple sclerosis (definite)</td>
<td>69</td>
</tr>
<tr>
<td>Multiple sclerosis (possible)</td>
<td>5</td>
</tr>
<tr>
<td>Others</td>
<td>43</td>
</tr>
<tr>
<td>Cervical myelopathy (spondylosis)</td>
<td>4</td>
</tr>
<tr>
<td>Meningitis</td>
<td>3</td>
</tr>
<tr>
<td>Migraine</td>
<td>3</td>
</tr>
<tr>
<td>Chronic inflammatory polyneuropathy</td>
<td>3</td>
</tr>
<tr>
<td>Conversion reaction</td>
<td>2</td>
</tr>
<tr>
<td>Guillain–Barré syndrome</td>
<td>2</td>
</tr>
<tr>
<td>Miscellaneous (one each—see text)</td>
<td>28</td>
</tr>
</tbody>
</table>

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The barbital electrophoresis buffer was 0.15 mol/L, pH 8.6, and contained 27 mmol of calcium lactate per liter. The fixing solution, 50 mmol of picric acid per liter of acetic acid (170 mL/L), was from Worthington Diagnostic Systems, Inc., Freehold, NJ 07728. The destaining solution consisted of absolute ethanol, glacial acetic acid, and distilled water (45/10/45, by vol). The staining solution, Coomassie Brilliant Blue R, 5 g/L, dissolved in the same solvent mixture is stable for at least six months. When in use, the stain should be discarded after two to three weeks or after having been used for 10–12 gels.

We used the LKB Multiphor and Power Supply and a Model ACD-18 densitometer (Gelman Sciences, Inc., Ann Arbor, MI 48106) for electrophoresis and quantification. Gelbond™ film, 85 × 100 mm, 0.2 mm thick (FMC Corp., Marine Colloids Division, Rockland, ME 04841) was the electrophoretic support. We used a Mylar foil (LKB) for sample applications.

Preparation of agarose plate. Pour 10 mL of a hot (90°C) 10 g/L solution of agarose in barbital buffer onto a Gelbond sheet, cut to fit a 94 × 84 mm glass plate; allow the solution to spread evenly to the edges. Allow to gel at room temperature and place it in a humid chamber at 4°C for at least 1 h before use.

Sample application and electrophoresis. After blotting the sample-application area of the gel with 1-cm wide filter paper, apply 3-μL samples of CSF, concentrated to contain about 20 g of protein per liter, and of serum, diluted fivefold with buffer, using a slotted sample application mask; allow the gel to absorb the samples for 7 to 10 min. Remove excess sample by gentle blotting and remove the mask. Electrophorese the gel at 9 V/cm for 15 min, then adjust to 10 V/cm and continue for 45 min. Fix the gel for 10 min by soaking it in picric acid solution, rinse it in absolute ethanol for 3–5 min, pressure-blot twice for 10 min each, and dry. Stain with Coomassie Brilliant Blue, rinse quickly in distilled water, and then destain by immersing the gel in destaining solution until a clear background and sharp bands are visible.

Calculations. The gamma-globulin and albumin fractions of CSF and serum were quantified by densitometry, and the γ-protein index was calculated for each patient. Based on data obtained for a concentrated CSF pool and a diluted normal serum, the day-to-day CV for the index was 12%. The reference interval (mean ± 2 SD) for the index was calculated from the normally distributed values of a control group.

Results

Figure 1 shows a representative electrophoretic pattern typical of those that formed the basis for all calculations. We calculated the upper limit of normal of 0.66 for the γ-protein index from the electrophoresis data (n = 48) on the control patients (patients without definite MS). Compared with 10 other studies in which immunochromatographic albumin and IgG assays were used (11, 16–24), our upper limit fell approximately in the middle of a range of 0.50 to 0.89. As shown in Figure 2, any shift of the upper limit of normal for the index <0.66 or >0.68 would increase the false-negative or false-positive rate, respectively. The mean index was 1.19 for our multiple sclerosis group and 0.44 for the control group. Half of the multiple sclerosis patients had an index >1.

We compared the data for both the oligoclonal band assay and the index with the clinical diagnoses. In 28/117 (24%) patients there was a disagreement between the indications of either the index or the oligoclonal bands and the clinical diagnosis (Table 2). These results were used to calculate sensitivity, specificity, and predictive values.

There were two false-positive index values (0.76 and 0.88) in the control group. The corresponding diagnoses were meningitis and neuropathy of unknown etiology. False-positive oligoclonal banding was seen in another four cases: cervical disc protrusion, spinal muscular atrophy, peritonitis, and Guillain–Barré syndrome. Thus the false-positive rates were 4% and 8%, respectively. Figure 2 shows the

![Figure 1. Typical electrophoretic pattern: Lanes 1,2: normal CSF and serum; lanes 3,4: abnormal CSF and corresponding serum](image)

![Figure 2. Distribution of results between the MS and the control groups: A,E: (+ bands, − index); B,F (− bands, − index); C,G (+ bands, + index); D,H (− bands, + index)

Horizontal line: upper limit of normal (this study, based on results for 48 subjects not having definite MS)](image)

<table>
<thead>
<tr>
<th>Table 2. Distribution of γ-Protein Index and Oligoclonal Bands</th>
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</thead>
<tbody>
<tr>
<td>Definite MS (n = 69)</td>
</tr>
<tr>
<td>+ Index and bands 47</td>
</tr>
<tr>
<td>+ Index only 3</td>
</tr>
<tr>
<td>+ Bands only 2</td>
</tr>
<tr>
<td>− Index and bands 17</td>
</tr>
<tr>
<td>Controls (n = 48)</td>
</tr>
<tr>
<td>+ Index and bands 0</td>
</tr>
<tr>
<td>+ Index only 2</td>
</tr>
<tr>
<td>+ Bands only 4</td>
</tr>
<tr>
<td>− Index and bands 42</td>
</tr>
</tbody>
</table>

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results for the index and for the band analyses in the two
groups.

In Table 3 we compare our values for sensitivity, specificity,
and predictive value with those found in previous
studies. In each of these studies the IgG index in patients
with definite MS was compared with the index in patients
with other diseases (control group). Table 4 illustrates the
value of using the two tests singly or together. The combina-
tion of an abnormal index and the presence of oligoclonal
bands increases the positive predictive value to 100%. In
Table 5 we suggest possibilities for appropriate interpretive
laboratory reporting when various combinations of bands
and index are encountered.

Discussion

Intrathecal synthesis of IgG can be demonstrated in about
90% of patients with MS. The extent of synthesis can be
estimated both by immunoassays and electrophoretic proce-
dures for qualitative assessment of CSF IgG. Newly adopted
diagnostic criteria for MS (26) include CSF IgG abnormali-
ties demonstrated by either immunoassay or electrophore-
sis.

Immunossays and electrophoretic assays provide com-
plementary information. Oligoclonal bands, which can be
visualized by the simple technique of electrophoresis of CSF
in agarose gels, are a sensitive indicator of intrathecal IgG
synthesis, and have been reported to be relatively stable
through the course of the disease (26). Quantification of CSF
and serum IgG and albumin by immunoassay, on the other
hand, provides quantitative information that may relate to
disease activity and provide a marker for response to
immunotherapy (27). Quantitative information about CSF
and serum IgG and albumin can also be derived from
densitometric tracings of dried agarose gel electrophore-
grams. Here we investigated the possibility of deriving both
qualitative and quantitative information about intrathecal
IgG synthesis from a single test, i.e., agarose gel electropho-
resis.

Ideally, a diagnostic test for MS should have very high
sensitivity and specificity. If this is not possible, however, a
test giving few or no false-positives (high specificity) is to be
preferred over one giving few false-negative values (high
sensitivity) (28). In our opinion the implications of a false-
positive diagnostic test in this clinical setting may be much
more damaging than those of a false-negative test. The γ-
protein index values we obtained after electrophoresis were
favorably matched with the IgG index results obtained by
other groups using separate immunological methods. The
results of the qualitative (i.e., oligoclonal bands) and quanti-
tative (i.e., index) analyses were generally concordant. That
the sensitivity of our oligoclonal band assay was lower than
in other studies may have resulted from any or all of the
following: (a) the conservative nature of our interpretations
(equivocal patterns were read as negative), (b) the compo-
nition of the control group, and (c) the degree of concentra-
tion of the CSF before electrophoresis. For example, if we had
used more-concentrated CSF, we could probably have de-
tected oligoclonal bands in a greater proportion of patients
with MS. However, for most diagnostic tests, improvements
in sensitivity are generally obtained at the expense of
specificity. In a recent study, for example, agarose electroph-
oresis of 80- to 100-fold-concentrated CSF samples had a
diagnostic sensitivity of 100% for definite MS but the
specificity was only 64% (29).

The observed false-positive rates of 4% and 8% for the γ-
protein index and oligoclonal bands, respectively, are con-
sistent with those in other studies (11, 13). The high positive
predictive value (96%, Table 3) of the index may reflect the
low number of patients in our study who had infections of
the central nervous system.

Diagnostic sensitivity improved when we used either the
presence of oligoclonal bands or a positive index to support
the clinical diagnosis of MS. Diagnostic specificity, however,
was greatly improved when both the presence of oligoclonal
bands and a positive index were used; not unexpectedly,
however, this combination decreased sensitivity, an accept-
able consequence from the clinical perspective. The com-
bination of positive oligoclonal bands and an index >1.0
strongly supports a suspected clinical diagnosis of MS,
syphilis, or central nervous system infection—entities that
can be differentiated by history, clinical signs and symp-
toms, and other laboratory measurements (7).

If the electrophoretic system is optimized, direct quantifi-
cation of the gamma and albumin fractions of CSF protein
can be used. Analysts must pay particular attention to sample
collection or dilution to assure consistency in the protein load
applied to the gel. Even more critical is the destaining step, to
assure a clear gel background and sharp bands, because place-
ment of markers separating the peaks for scanning must be made
on a flat baseline.

Table 3. Comparison of Immunologic Quantification of the
IgG Index and Densitometric Quantification of the
γ-Protein Index

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>IgG index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olson and Pettersson (11)</td>
<td>88</td>
<td>79</td>
<td>43</td>
<td>97</td>
</tr>
<tr>
<td>Hershey and Trotter (13)*</td>
<td>91</td>
<td>85</td>
<td>74</td>
<td>92</td>
</tr>
<tr>
<td>Link and Tibbling (9)</td>
<td>86</td>
<td>90</td>
<td>71</td>
<td>96</td>
</tr>
<tr>
<td>Caroscio et al. (30)</td>
<td>94</td>
<td>73</td>
<td>48</td>
<td>98</td>
</tr>
<tr>
<td>γ-Protein index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This study</td>
<td>73</td>
<td>96</td>
<td>96</td>
<td>71</td>
</tr>
</tbody>
</table>

*Excludes definite MS patients who were taking steroids.

Table 4. Comparison of Oligoclonal Bands and the γ-
Protein Index Calculated by Densitometry

<table>
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</thead>
<tbody>
<tr>
<td>γ-Protein index</td>
<td>73</td>
<td>96</td>
<td>96</td>
<td>71</td>
</tr>
<tr>
<td>Oligoclonal bands</td>
<td>71</td>
<td>92</td>
<td>93</td>
<td>69</td>
</tr>
<tr>
<td>Bands and index</td>
<td>68</td>
<td>100</td>
<td>100</td>
<td>71</td>
</tr>
<tr>
<td>Bands or index</td>
<td>75</td>
<td>71</td>
<td>90</td>
<td>69</td>
</tr>
</tbody>
</table>

Table 5. Suggested Interpretations for Oligoclonal
Bands and γ-Protein Index

<table>
<thead>
<tr>
<th>Oligoclonal bands</th>
<th>γ-Protein index</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>−</td>
<td>No abnormality found</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>Supports clinical diagnosis of MS</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>Supports clinical diagnosis of MS</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Strongly supports clinical diagnosis of MS</td>
</tr>
</tbody>
</table>

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be essential when visual interpretation is borderline or when finer discrimination is necessary.

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References