Detection of Oligoclonal Immunoglobulins in Cerebrospinal Fluid by an Immunofixation-Peroxidase Method

Sophie Richard, Véronique Miossec, Jean-François Moreau, and Jean-Luc Taupin*

Background: The detection of intrathecal synthesis of immunoglobulins is used in the diagnosis of multiple sclerosis (MS). We tested the semiautomated immunofixation-peroxidase (IFPOD) technique, which uses high-resolution agarose gel electrophoresis (HRAGE) directly followed by immunofixation with a peroxidase-labeled anti-IgG antiserum to detect oligoclonal immunoglobulins in cerebrospinal fluid (CSF).

Methods: We analyzed 230 consecutive matched serum/CSF pairs that arrived in the laboratory over a 6-month period with both IFPOD and our routine techniques, immunofixation electrophoresis (IFE) and HRAGE. For IFPOD, CSF samples were not concentrated before testing.

Results: Among the 230 samples were 12 clinically definite MS, 33 clinically probable, and 20 clinically possible MS samples. IFPOD and HRAGE/IFE each detected oligoclonal IgG in CSF in 10, 16, and 7 cases of these respective groups. For clinically definite MS, sensitivity and specificity (95% confidence intervals) were, respectively, 83% (51–97%) and 79% (73–84%).

Conclusions: The IFPOD technique performs comparably to other analytical methods, without the requirement for sample concentration, and may represent an attractive alternative in testing for intrathecal immunoglobulin synthesis.

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Several disorders of the central nervous system (CNS), such as multiple sclerosis (MS) and other CNS inflammatory diseases, are associated with an increase in protein concentration in the cerebrospinal fluid (CSF), which can be attributable to an altered permeability of the blood–brain barrier, to intrathecal synthesis of immunoglobulins, or to a combination of both. In this context, the examination of the CSF includes quantitative measurements of immunoglobulin and albumin concentrations and a qualitative analysis of immunoglobulins in comparison with the serum. The immunochemical detection in the CSF of an oligoclonal profile in the IgG fraction, which is absent in the serum, remains the major biological diagnostic marker for MS because it indicates intrathecal synthesis of IgG. Although intrathecal synthesis of IgA and/or IgM may also be detected in MS, they are found less frequently than IgG and are more commonly found in infectious CNS diseases (1–4).

To date, the reference method for the detection of oligoclonal bands (OCBs) is isoelectric focusing (IEF) (5). Nevertheless, other techniques exist, such as agarose gel electrophoresis (AGE) and immunofixation electrophoresis (IFE) (6, 7), which are performed with concentrated CSF to improve sensitivity. In our laboratory, the detection of OCBs in CSF is routinely performed with two methods: high-resolution AGE (HRAGE) to compare serum and CSF, and IFE, which is performed on the CSF sample with antisera specific for heavy chain and κ and λ light chains. In this study, we tested a new technique consisting of IFE performed on a high-resolution agarose gel and coupled to immunoenzymatic detection of the immunoglobulin complexes directly on the gel. This technique, which is called immunofixation-peroxidase (IFPOD), is semiautomated on the “Hydrasys” apparatus distributed by Sebia and does not require a concentration step for the CSF sample. The purpose of the present investigation was to compare the performance of the IFPOD technique with other methods.

1 Nonstandard abbreviations: CNS, central nervous system; MS, multiple sclerosis; CSF, cerebrospinal fluid; OCB, oligoclonal band; IEF, isoelectric focusing; IFE, immunofixation electrophoresis; HRAGE, high-resolution agarose gel electrophoresis; IFPOD, immunofixation-peroxidase; CDMS, clinically definite MS; CPMS, clinically probable MS; PMS, possible MS; CNSID, CNS inflammatory disease; CNSNID, CNS noninflammatory disease; PNSD, peripheral nervous system disease; and CS, control sample.
method with that of HRAGE + IFE for the detection of OCBs in CSF.

Materials and Methods

Patients
We analyzed prospectively 230 consecutive matched pairs of CSF and serum samples drawn between November 1999 and April 2000 from patients hospitalized for neurologic disorders in the four departments of neurology of the Bordeaux University Hospitals. The study was approved by the institution’s ethics committee. The clinical diagnosis of MS followed the criteria defined by Poser et al. (8). Clinically definite MS (CDMS) is defined by the occurrence of (a) two attacks and clinical evidence of two lesions or (b) two attacks and clinical evidence of one lesion plus test evidence (such as magnetic resonance imaging or evoked potentials) of a second lesion. Clinically probable MS (CPMS) is defined as one attack plus clinical evidence of one lesion and test evidence of one lesion. Clinically possible MS (PMS) is defined as CPMS except that the test evidence was not affirmative.

Biological Samples
CSF and blood were obtained by lumbar puncture and venipuncture, respectively. Blood samples were drawn without anticoagulant and centrifuged (300g for 10 min at 4 °C), and the serum was harvested. All samples were stored for up to 4 days at 4 °C before being processed.

Determination of Total and Specific Protein Concentrations
The total protein concentration in unconcentrated CSF was determined according to the method of Bradford (9) with the Total Protein Test reagent set (Bio-Rad). An external control (Liquicheck Spinal Fluid Control), also from Bio-Rad, was added. Albumin, IgG, IgA, and IgM concentrations were measured in serum and unconcentrated CSF by immunonephelometry using a BNII apparatus (Dade-Behring) with calibrators and internal controls provided by Dade-Behring and according to the manufacturer’s recommendations. An external control (Liquicheck Immunology Control; Bio-Rad) was also used for these analytes in serum. As a mirror of blood–CSF barrier status, the CSF/serum albumin ratio was calculated; its threshold of positivity varied between $6 \times 10^{-3}$ and $8 \times 10^{-3}$ with the age of the patients. To detect intrathecal synthesis of IgG (ISI), we calculated the IgG/albumin index (10), the Tourtelotte index (11), the Reiber index (12), and the Schuller index (13). The IgG/albumin index was calculated by the following formula:

$$\text{IgG/Albumin index} = \frac{\text{IgG}_{\text{CSF}}/\text{IgG}_{\text{serum}}}{(\text{albumin}_{\text{CSF}}/\text{albumin}_{\text{serum}})}$$

and was positive above 0.65. The Tourtelotte index was defined by the following formula:

$$\text{ISI (mg/24h) = [IgG}_{\text{CSF}} - (IgG}_{\text{serum}}/369)$$
$$- [(\text{albumin}_{\text{CSF}} - \text{albumin}_{\text{serum}})/230]$$
$$\times (\text{IgG}_{\text{serum}}/\text{albumin}_{\text{serum}}) \times 0.215$$

The Reiber index was defined by the following formula:

$$\text{ISI (mg/L) = [IgG}_{\text{CSF}} - \{0.8}$$
$$\times [(\text{albumin}_{\text{CSF}}/\text{albumin}_{\text{serum}})^2 + 15.10^{-6}]^{0.5}$$
$$- 18.10^{-4}] \times \text{IgG}_{\text{serum}}$$

The Schuller index was defined by the following formula:

$$\text{ISI (mg/L) = IgG}_{\text{CSF}} - \{30 + [(\text{albumin}_{\text{CSF}} - 240)/60}$$
$$\times \text{IgG}_{\text{serum}}$$

Intrathecal synthesis of IgG is suspected when the result is $>3.5\text{ mg/24 h}$ for the Tourtelotte index and $0\text{ mg/L}$ for the Reiber index and the Schuller index.

HRAGE and IFE
For electrophoretic analysis of the immunoglobulin profile, an aliquot of the CSF specimen was concentrated in a Minicon B-15 concentrator (Amicon) to reach an IgG concentration of 2.5 g/L. This usually corresponded to a 20- to 80-fold concentration. For HRAGE, we used the high-resolution agarose gel reagent set “Hydragel–mini HR” (Sebia) according to the manufacturer’s recommendations. Serum samples were diluted 1:10 in 9 g/L NaCl. Matched CSF and serum specimens (5 μL of each) were loaded on the agarose gel. After electrophoresis at 80 V for 45 min, the gel was washed in saline buffer and fixed in 600 mL/L ethanol–300 mL/L deionized water–100 mL/L acetic acid for 15 min. The gel was dried, stained in acid violet for 5 min, and then destained in two successive baths of destaining solution (Sebia) until the background became completely colorless and clear. Finally, the gel was dried.

IFE was performed using the agarose gel reagent set “Hydragel IF” (Sebia), according to the manufacturer’s recommendations. Concentrated CSF samples were diluted 1:3 for the analysis of IgG and κ chains and 1:2 for the analysis of λ chains. Samples (5 μL) were loaded in the slit of a template adjusted over three gel tracks. The electrophoresis was then performed for 35 min at 100 V. After electrophoresis, an antiserum loading template was aligned over the gel, and 75 μL of anti-γ heavy chain, anti-κ light chain, or anti-λ light chain antiserum was added in one of the three lanes. After incubation for 10 min, the excess antiserum was removed by applying one thin filter to the surface of the gel. The gel was washed in washing buffer (Sebia) and pressed twice for 10 and 20 min, respectively. The gel was then dried for 5 min, stained in acid violet for 5 min, destained, and finally dried.
IFPOD

We used the “Hydragel 6-CSF” reagent set and the Hydrasys semiautomated electrophoresis apparatus (both from Sebia), according to the manufacturer's recommendations. Before the analysis, the CSF and serum samples to be compared were adjusted at an IgG concentration of 10 mg/L. Usually for the CSF this corresponded to a 1:2 to 1:100 dilution, meaning that this technique did not require concentration of the CSF before the analysis. One gel is designed to analyze six matched CSF/serum pairs with one antiserum. CSF and serum specimens (15 μL of each) were loaded side by side with a specifically designed applicator system to the surface of a 0.8% high-resolution agarose gel. After separation by electrophoresis under a constant 20 W at 20 °C controlled by the Peltier effect (80 V-h delivered during ~17 min of migration), IgG was specifically immunoprecipitated in the gel for 10 min by the addition of 50 μL of peroxidase-labeled anti-IgG antiserum, diluted 1:11 with a specific antiserum diluent, to each lane of the gel with an adapted loading template. The excess antiserum was then removed by blotting with a filter paper. The gel was then washed twice, and the immunoprecipitated complexes were revealed directly in the gel by incubation with the POD substrate TTF3 in the presence of hydrogen peroxide for 15 min at 30 °C. The substrate was transformed into a pink product that directly precipitated into the gel. The gels were then washed once again to remove the excess of substrate and dried.

INTERPRETATION OF GELS AND STATISTICAL ANALYSIS

Interpretation was based on observation by three clinical biologists (two involved daily in the interpretation of these gels, and one involved occasionally) of the profiles obtained for the serum and the CSF samples for all the techniques; observers looked for a mono- or oligoclonal IgG pattern. The HRAGE + IFE reference method was applied to all samples. The gels were analyzed by the biologists independently of one another. To avoid any bias in the interpretation, the different techniques were considered separately to avoid reviewer bias as follows. Because HRAGE + IFE was our diagnostic method, these gels were analyzed with the shortest delay to rapidly give a result to the physician. The IFPOD gels were kept apart and analyzed at the end of the study. In our study, a positive result was defined as the detection of at least one immunoglobulin band in the CSF sample that was absent in the serum sample, therefore providing evidence of intrathecal synthesis. Gels presenting a discordant interpretation among the biologists were reanalyzed by the two senior biologists until an agreement was reached. The sensitivity and specificity of IFPOD and HRAGE + IFE were calculated. Overall agreement between the methods and between the observers was assessed by the Cohen κ statistical test. The study was conducted according to previously published guidelines for CSF analysis and interpretation (5, 14).

RESULTS

CLASSIFICATION OF PATIENTS

We analyzed the 230 consecutive matched pairs of CSF and serum samples that arrived in the laboratory between November 1999 and April 2000 from patients hospitalized for neurologic disorders in the four departments of neurology of the Bordeaux University Hospitals. We considered the diagnosis given from the clinical parameters before the biological exploration. Among the 230 patients studied, 65 patients were included in the group of MS patients according to the criteria delineated by Poser et al. (8), which was subdivided in CDMS (12 patients), CPMS (33 patients), and PMS (20 patients). Forty-four patients had another kind of CNS inflammatory disease (CNSID), 86 had a CNS noninflammatory disease (CNSNID), 24 had peripheral nervous system disease (PNSD), and 11 did not fit in any of these groups [control samples (CS): patients with fatigue syndrome, severe headaches, dizziness, or syncope; Table 1]. As is common in MS [see Refs. (15, 16) for review], we noticed that patients with CDMS or suspected of having MS (CPMS and PMS groups) were younger and were women in the majority of cases (Table 1).

DETECTION OF OLIGOCLONAL IgG BY IFPOD

The matched pairs of CSF and serum were analyzed with the IFPOD technique to study the pattern of the IgG fraction. Examples of the profile routinely obtained with this method are given in Fig. 1. On this gel, matched serum and CSF sample pairs 1, 3, and 6 showed a polyclonal pattern without any extra band in either the serum or in the CSF. The samples corresponded to patients suffering from polyneuropathy (patient 1), neuronal degeneration (patient 3), and papillary edema (patient 6). In contrast, the CSF samples in matched sample pairs 4 and 5 presented an oligoclonal pattern with three and four clearly visible bands, respectively, whereas the serum samples showed a complete absence of bands. Both patients had CDMS. Matched sample pair 2 presented one identical monoclonal band in both the serum and CSF, and this patient had a myeloma with polyradiculoneuropathy. This pattern is consistent with leakage of the monoclonal immunoglobulin from the blood across the blood–brain barrier.

Table 1. Distribution of the patients according to disease, sex, and age.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Age, mean ± SD, years (no. of patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Men</td>
</tr>
<tr>
<td>CDMS</td>
<td>12</td>
<td>36 ± 2.08 (3)</td>
</tr>
<tr>
<td>CPMS</td>
<td>33</td>
<td>41 ± 16.1 (14)</td>
</tr>
<tr>
<td>PMS</td>
<td>20</td>
<td>41 ± 14.5 (5)</td>
</tr>
<tr>
<td>CNSID</td>
<td>44</td>
<td>50 ± 19.9 (26)</td>
</tr>
<tr>
<td>CNSNID</td>
<td>86</td>
<td>61 ± 20.3 (50)</td>
</tr>
<tr>
<td>PNSD</td>
<td>24</td>
<td>60 ± 11.8 (11)</td>
</tr>
<tr>
<td>CS</td>
<td>11</td>
<td>58 ± 10.9 (6)</td>
</tr>
</tbody>
</table>
Simultaneously with the IFPOD analysis, all patients’ CSF/serum pairs were also analyzed by IFE and HRAGE, which are our two routine techniques for MS diagnosis. IFE, which is slightly more sensitive than HRAGE for the detection of oligoclonal banding [Ref. (6) and our personal results], is used to analyze concentrated CSF, and HRAGE is used to compare concentrated CSF with the serum. The results obtained with the current strategy were compared with those obtained with the IFPOD method. As an example, the results for two different pairs of samples are displayed in Figs. 2 and 3. Fig. 2 displays the result obtained for samples from a 37-year-old woman suffering severe headaches, who was a member of the control group. The CSF required 80-fold concentration before analysis by IFE and HRAGE and 1:3 dilution for the IFPOD analysis. The results of the IFE and the HRAGE (Fig. 2A) did not show any detectable mono- or oligoclonal bands in either the serum or the CSF. Similar results were obtained with the IFPOD technique (Fig. 2B). Fig. 3 was obtained with samples from a 23-year-old woman presenting with PMS. The CSF was concentrated 60-fold before analysis by IFE and HRAGE and diluted 1:4 for the IFPOD analysis. With IFE, four IgG bands, two of the \( \kappa \) isotype and two of the \( \lambda \) isotype, were detected in the CSF. With HRAGE, one strong band was visible in the CSF sample, which was absent from the serum sample (Fig. 3A). With the IFPOD method, an oligoclonal profile consisting of five IgG OCBs was clearly identified (Fig. 3B). These examples show that although the CSF samples very often need to be diluted before analysis by IFPOD, instead of being concentrated, the sensitivity is such that the technique appears to be able to clearly detect the oligoclonal pattern whenever it is present.

**PERFORMANCE COMPARISON OF IFPOD AND HRAGE + IFE**

Comparative interpretation of the profiles obtained for the 230 matched pairs of samples was carried out independently by three clinical biologists who analyzed the different techniques separately. Intrathecal synthesis (i.e., a positive result) was defined by the detection of one or several immunoglobulin bands in the CSF sample that were not found in the matched serum sample. A negative result was defined by the absence of IgG band(s) in the CSF or the presence of IgG band(s) that were also found in the serum.

Among the 230 samples analyzed, discrepancies in the interpretation occurred among the three biologists in only eight cases: seven cases analyzed by HRAGE + IFE and one analyzed by IFPOD. Between-observer variability was therefore very low, as confirmed by the calculation of the Cohen \( \kappa \) statistical test (\( \kappa = 0.94 \) and 0.98 for HRAGE + IFE and IFPOD, respectively). For these samples, a consensus was reached after a second analysis; therefore, no indeterminate test result was considered in the statistical analysis. Among the 230 specimens, 178 (77.4%) were negative by both IFPOD and HRAGE + IFE. Mono- or oligoclonal bands were detected in CSF only for 52 (22.6%) specimens. Among these 52 CSF specimens, 46 (88.5%) were positive by both methods, 5 (9.6%) by IFPOD alone, and 1 (1.9%) by HRAGE + IFE alone (Table 2).

The distribution of the presence or absence of immunoglobulin bands according to the clinical group is shown in Table 3. In CDMS, CPMS, and PMS, there were no differences in the detection of OCBs by both methods in...
CSF because the same samples were positive with the two methods, corresponding to 83.3%, 48.5%, and 35% of the patients, respectively. For the five samples positive by IFPOD only, the banding pattern was always faint, and in four cases, it was limited to only one band [one CNSID (Guillain-Barré syndrome) and three CNSNIDs (one senile dementia, one epilepsy, and one amyotropic lateral sclerosis)] or to two bands [one CNSID (Guillain-Barré syndrome)]. To illustrate this, the gels obtained with the various methods for two of these patients are depicted in Fig. 4. For patient A (top panel) and patient B (bottom panel), one faint band and two faint bands, respectively, could be seen in the CSF with IFPOD only. The sample that was found positive by HRAGE + IFE only consisted of a monoclonal free κ chain, which could therefore not be detected with the IFPOD method in the conditions used. In addition, two samples displayed one single visible band with both IFPOD and HRAGE + IFE, which corresponded to one CNSID (Listeria-associated meningoradiculitis) and one PNSD (myasthenia). Therefore, although seven CSF samples were found to contain only one band (Table 3), none of them belonged to the patients with MS.

Statistical analysis showed that for CDMS vs all others patient groups, the sensitivities (95% confidence interval) of the IFPOD and HRAGE + IFE methods were identical at 83% (51–97%). The specificity of the IFPOD method was 79% (73–84%) and was almost identical to that of the HRAGE + IFE method, which was 81% (75–86%). Overall agreement between the two methods was very good (κ = 0.92) as determined by the Cohen κ statistical test. Therefore, this study showed that these techniques did not differ significantly for the detection of intrathecal IgG synthesis in the CDMS patients. The detection of OCBs by any of these methods is more sensitive than the calculation of the CSF/serum albumin ratio, which assesses blood–brain barrier permeability, or any of the formulas estimating intrathecal immunoglobulin synthesis, such as the IgG/albumin index, the Tourtelotte equation, the Schuller equation, and the Reiber equation, which detected an anomaly in only 58.3% of the CDMS patients (Table 4).

### Table 2. Distribution of the 230 samples analyzed with (positive) or without (negative) immunoglobulin banding in CSF only, for IFPOD vs HRAGE + IFE.

<table>
<thead>
<tr>
<th>IFPOD</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>46</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>178</td>
</tr>
</tbody>
</table>

### Table 3. Detection of intrathecal synthesis of immunoglobulins, depending on the presence or absence of immunoglobulin banding in CSF, for each group of patients and each method used.

<table>
<thead>
<tr>
<th>Group (no. of patients)</th>
<th>IFPOD</th>
<th>HRAGE + IFE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presence/Absence of CSF banding (% positive samples)</td>
<td>Presence/Absence of CSF banding (% positive samples)</td>
</tr>
<tr>
<td>CDMS (12)</td>
<td>10/2 (83.3)</td>
<td>10/2 (83.3)</td>
</tr>
<tr>
<td>CPMS (33)</td>
<td>16/17 (48.5)</td>
<td>16/17 (48.5)</td>
</tr>
<tr>
<td>PMS (20)</td>
<td>7/13 (35)</td>
<td>7/13 (35)</td>
</tr>
<tr>
<td>CNSID (44)</td>
<td>9/35 (20.5) [2]a</td>
<td>8/36 (18.2) [1]a</td>
</tr>
<tr>
<td>CNSNID (86)</td>
<td>7/79 (8.1) [3]a</td>
<td>4/82 (4.7)</td>
</tr>
<tr>
<td>PNSD (24)</td>
<td>2/22 (8.3) [1]a</td>
<td>2/22 (8.3) [1]a</td>
</tr>
<tr>
<td>CS (11)</td>
<td>0/11 (0)</td>
<td>0/11 (0)</td>
</tr>
</tbody>
</table>

a Number of patients with only one band.

### Detection of IgA and IgM by IFPOD

In some cases, it can be interesting to detect intrathecal synthesis of immunoglobulins belonging to the IgA or IgM isotypes. As currently performed in the laboratory, IFE allows for the detection of isotypes other than IgG by use of anti-κ and anti-λ antisera in addition to the HRAGE. In such cases, identification is pursued by a new IFE performed with anti-IgA and anti-IgM antisera. In the case of IFPOD, labeled anti-IgA and anti-IgM have also been developed, and Fig. 5 demonstrates that they are...
suitable for detecting abnormal IgA and IgM in CSF without preliminary concentration because the only requirement to meet for the assay is an antibody concentration adjusted to 10 mg/L. Fig. 5A shows the case of an 81-year-old woman presenting with Waldenström disease and axonal neuropathy. The IFPOD gel showed a monoclonal IgM band in serum that was also found in CSF. Fig. 5B shows the case of a 78-year-old man with IgA myeloma. The IFPOD gel showed a monoclonal IgA band in the serum that was also found in the CSF.

Discussion

The detection of intrathecal synthesis of IgG is a widely used diagnostic marker for MS. Our goal was to compare the performance of a new technique, IFPOD, with that of HRAGE combined with IFE, which are the diagnostic techniques used jointly in our laboratory. We analyzed 230 consecutive matched serum/CSF pairs that arrived in the laboratory over a 6-month period and that covered a variety of neurologic disorders of central or peripheral origin, either inflammatory or not (see Table 1). For MS, the demographic characteristics of our patients were in accordance with previously reported studies [reviewed in Refs. (15, 16)].

The different techniques were applied blindly to all samples in the study, all of which were considered for statistical interpretation of the results. To avoid reviewer bias, IFPOD gels were analyzed at the end of the study, whereas HRAGE + IFE gels were analyzed as soon as possible after they were run to give the physician a result with the shortest delay. Because our goal was to assess the technical performance of the IFPOD method with regard to the detection of any abnormal CSF IgG pattern, in this study we considered as positive samples showing at least one immunoglobulin band in CSF only, although the clinical significance of the CSF IgG profile for the diagnosis of MS is reached when an oligoclonal pattern (i.e., at least two bands) can be documented. However, none of the seven samples displaying only one band belonged to patients in the CDMS, CPMS, or PMS groups. This study shows that IFPOD displayed sensitivities and specificities very close to those of HRAGE + IFE for the detection of intrathecal synthesis of IgG in MS. Discrepancies occurred in only 6 of 230 cases. In five cases, IFPOD showed intrathecal synthesis, whereas HRAGE + IFE did not. This involved patients with CNS diseases other than MS: two had CNSID and three had CNSNID. Interestingly, four of these patients displayed only one detectable IgG band, whereas one showed two bands. In all five cases, the bands were always faint, suggesting that IFPOD is more sensitive than HRAGE + IFE. This was confirmed by the fact that IFPOD is performed with unconcentrated CSF, which moreover needs to be diluted in most cases because the required concentration of 10 mg/L for IgG is usually already reached in CSF of healthy individuals. As a consequence, a smaller volume of CSF is necessary, and the absence of the concentration step helps avoid additional pitfalls, such as loss of proteins in the filter or nonspecific aggregation of immunoglobulins, giving the IFPOD technique a great advantage over HRAGE and IFE. Because of the enzymatic signal amplification used in the IFPOD, the IgG bands on the gels were often more numerous and/or more sharply defined than the bands seen with IFE or HRAGE.

In our study, for one case, both HRAGE and IFE were positive and showed one band in CSF only, which corresponded to a free κ light chain. As expected, this band could not be detected with the IFPOD method, which was performed with an IgG-specific antiserum. This could be of importance because several reports have highlighted that free light chains could be valuable in supporting the diagnosis (17, 18). However, in most laboratories, the detection of OCBs in CSF is limited to IgG, regardless of the technique used. Therefore, such positive samples can be missed with the IFPOD method. Labeled anti-κ and anti-λ antisera are being developed by Sebia, but we have not yet tested these reagents. Such reagents could be

<table>
<thead>
<tr>
<th>Clinical group (no. of patients)</th>
<th>Albumin ratio above threshold, n (%)</th>
<th>IgG/Albumin index &gt;0.65, n (%)</th>
<th>Tourtelotte index &gt;3.5 mg/24 h, n (%)</th>
<th>Schuller index &gt;0 mg/L, n (%)</th>
<th>Reiber IgG index &gt;0 mg/L, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDMS (12)</td>
<td>4 (33.3)</td>
<td>7 (58.3)</td>
<td>7 (58.3)</td>
<td>7 (58.3)</td>
<td>7 (58.3)</td>
</tr>
<tr>
<td>CPMS (33)</td>
<td>12 (36.3)</td>
<td>13 (39.4)</td>
<td>11 (33.3)</td>
<td>13 (39.4)</td>
<td>13 (39.4)</td>
</tr>
<tr>
<td>PMS (20)</td>
<td>8 (40)</td>
<td>6 (30)</td>
<td>6 (30)</td>
<td>6 (30)</td>
<td>5 (25)</td>
</tr>
</tbody>
</table>

Table 4. Blood–CSF barrier permeability abnormalities and quantitative measurement of intrathecal IgG synthesis assessed by the albumin ratio, the IgG/albumin index, the Tourtelotte index, the Schuller index, and the Reiber index.

Fig. 5. Detection of IgA and IgM with IFPOD.

CSF and serum (S) from patients with a monoclonal IgM (A) or a monoclonal IgA (B) were adjusted to 10 mg/L for IgG and for IgM or IgA and assayed by IFPOD.
routinely used in addition to the anti-IgG antiserum to ascertain that all immunoglobulin isotypes will be detected. In the case of a positivity with either of anti-κ or anti-λ antiserum, the subsequent use of the anti-IgA and anti-IgM antiseras will help to identify the isotypes of the non-IgG bands, as we use with IFE in our laboratory. However, IgM and/or IgA are detected far less frequently in MS than is IgG, and have not demonstrated to be of diagnostic value (1, 19, 20).

IEF with immunoblotting of IgG is the recommended method at present for CSF analysis (5). According to several published reports, it allows the detection of oligoclonal banding in CSF in 88–95% of patients with CDMS (21–23). Our study suggests that the sensitivity of the IFPOD method is very close to that of IEF because it allowed detection of an IgG oligoclonal profile in 83% of the patients who belonged to this group. However, given the small number of samples analyzed (n = 12), this needs to be confirmed in a larger series of patients.

Another point to be considered is the practicability of the assay. IEF is time-consuming and difficult to interpret, and the absence of standardization hampers it. It requires specific skills and material and may be difficult to set up and maintain in a laboratory that is not frequently involved in CSF analysis. In contrast, the IFPOD method is in most cases easy to interpret, is completed in <2.5 h, is semiautomated, and presents the advantage of using standardized reagents. In addition, the use of IFPOD is cost-effective because its cost is almost identical to that of HRAGE, and is one-half that of IFE.

In conclusion, we think that the IFPOD method represents a reasonable alternative for detection of OCBs in CSF.

We thank the technical staff of the immunology laboratory of the CHU de Bordeaux for their contribution to this work, the medical staff of the four departments of neurology of the CHU de Bordeaux for their participation, and Dr. Catherine Verret (ISPED, University of Bordeaux II, Bordeaux, France) for helpful advice in the statistical interpretation of the results.

References


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