Early Differential Diagnosis of Multiple Sclerosis Using a New Oligoclonal Band Test

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Background: Intrathecal IgG synthesis (ITGS), in conjunction with magnetic resonance imaging, can help in the early diagnosis of multiple sclerosis (MS). Recently, we developed a new oligoclonal IgG band (OCGB) test for ITGS detection that is more sensitive and easier to interpret than previously described methods.

Objective: To assess the accuracy of a new OCGB detection test in the diagnosis of MS.

Design: Prospective observational study.

Setting: A hospital neurology department.

Patients: A total of 385 patients with various neurologic disorders.

Main Outcome Measures: The sensitivity and specificity of the OCGB detection test for MS diagnosis.

Results: Intrathecal IgG synthesis was found in 127 patients with MS (96.2%), 18 (35.3%) with central nervous system infections, and 1 with motor neuron disease. Two patterns reflected ITGS. One pattern, showing OCGBs restricted to cerebrospinal fluid, was predominantly found in MS. The other pattern, with OCGBs in serum and additional bands in cerebrospinal fluid, was mostly found in central nervous system infections. No patients with other inflammatory neurologic diseases showed ITGS. These patients frequently displayed a mirror pattern, with identical bands in serum and cerebrospinal fluid. Considering all patients, the sensitivity for the diagnosis of MS was 96.2%, and the specificity was 92.5%. Excluding infections, which usually do not present a differential diagnosis problem with MS, the sensitivity was still 96.2%, and the specificity increased to 99.5%.

Conclusion: The accuracy of this OCGB method reinforces the value of cerebrospinal fluid studies in the early differential diagnosis of MS.

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Multiple sclerosis (MS) is the most frequent demyelinating disease of the central nervous system (CNS). The rationale for MS treatment is to reduce the disease activity and to delay the progression of disability. Thus, an early and accurate diagnosis of MS is important because it has been shown that early treatment has a beneficial effect on disease evolution.1,2

Because no single test provides a definite diagnosis, different criteria have been used for MS diagnosis.3,4 Different paraclinical tests, such as detection of intrathecal IgG synthesis (ITGS) (present in most patients with MS), evoked potentials, and imaging techniques, are used to support the diagnosis when necessary. Intrathecal IgG synthesis determined by oligoclonal IgG band (OCGB) detection is an important tool for MS diagnosis.5 We developed a novel OCGB assay that consists of isoelectric focusing (IEF) and IgG immunodetection by an alkaline phosphatase–labeled anti-IgG antibody. It yields high sensitivity and a sharp and strong pattern of bands that is easy to interpret.7 Alkaline phosphatase has proved to be useful in the detection of oligoclonal IgM bands8 that have an important role as prognostic markers in MS.9,10

We herein analyze the presence of OCGBs using the alkaline phosphatase method in 385 patients with different neurologic diseases to assess the validity of this test for MS diagnosis. Special attention was paid to other inflammatory neurologic diseases of the CNS that may represent important problems in the differential diagnosis of the disease.
METHODS

PATIENTS

A total of 385 patients studied in the Neurology Department of Ramón y Cajal Hospital were included in this study. Patients were divided into 6 groups. Group 1 comprised 132 patients with clinically definite MS according to the criteria of Poser et al. We further divided this group into 27 patients who underwent lumbar puncture during the first attack of the disease and converted to clinically definite MS during follow-up (group 1A) and 105 patients with a clinically definite MS diagnosis at lumbar puncture (group 1B). Group 2 comprised 37 patients with inflammatory diseases of the CNS different from MS, including 9 with myelitis, 8 with CNS vasculitis, 4 with neuro lupus, 4 with paraneoplastic syndrome, 3 with Behçet disease, 3 with Rasmussen disease, 2 with Hashimoto encephalitis, 2 with gluten ataxia, 1 with neurosarcoidosis, and 1 with Sjögren disease. Group 3 was composed of 26 patients with inflammatory diseases of the peripheral nervous system: 15 with Guillaume-Barré syndrome and 11 with other inflammatory polyneuropathies (9 with chronic inflammatory demyelinating polyneuropathy and 2 with monoclonal IgM-associated polyneuropathy).

Group 4 comprised 100 patients with noninflammatory neurologic diseases: 31 with neurodegenerative disorders (16 with Alzheimer disease, 4 with frontotemporal dementia, 1 with dementia with Lewy bodies, 9 with motor neuron disease, and 1 with Parkinson disease), 24 with stroke, 12 with neoplastic meningitis, 11 with pseudotumor cerebri, 7 with normal pressure hydrocephalus, 6 with diabetic polyneuropathy, 4 with olivopontocerebellar atrophy, 2 with arteriovenous malformation, 2 with idiopathic epilepsy, and 1 with Wernicke disease. Group 5 consisted of 51 patients with CNS infections: 30 with acute viral meningoencephalitis, 4 with acute bacterial meningitis, 4 with tuberculous meningitis, 1 with neosiphyllis, 1 with spinal cord schistosomiasis, 1 with progressive multifocal leukoencephalopathy, and 10 with human immunodeficiency virus infection. 3 of them with superimposed tuberculous meningitis and 2 others with progressive multifocal leukoencephalopathy. Group 6 comprised 39 patients with nonspecific headaches without any neurologic abnormalities.

SAMPLES AND LABORATORY TESTS

Paired serum and cerebrospinal fluid (CSF) samples were collected for diagnostic purposes after informed consent was obtained. The samples were stored at –40°C until oligoclonal band testing was performed.

Serum and CSF IgG concentrations were analyzed in a nephelometer (BNII; Behring, Marburg, Germany). Oligoclonal IgG detection was performed by IEF and immunodetection as previously described. Before IEF, serum samples were diluted in deionized water to reach the same IgG concentration as that of parallel CSF samples. Isoelectric focusing was performed on agarose gels prepared with agarose IEF, Pharmalyte pH 3-10, and Pharmalyte pH 8-10.5 (all from Amersham Biosciences, Uppsala, Sweden). Focusing was performed on a Multiphor II apparatus (Amersham Biosciences) cooled to 10°C. The electrode strips were soaked with 1M sodium hydroxide (catholyte) and 0.05M sulfuric acid (anolyte). Three-microliter paired samples were applied on the anodic side of the gel. After IEF, the proteins were transferred to a nitrocellulose membrane (Millipore, Billerica, Mass). The nitrocellulose membrane was blocked in 2% dry milk in saline serum for 30 minutes and then incubated with alkaline phosphatase–conjugated rabbit anti-human IgG (The Jackson Laboratory, Bar Harbor, Me) diluted 1:5000 in 0.2% dry milk in saline for 1 hour at room temperature in a platform shaker. The membrane was then washed 25 times with tap water and once with saline serum for 10 minutes. Finally, the membrane was stained using nitro blue tetrazolium and bromo-chloroindolyl phosphate.

Figure. Four different patterns were found in IgG immunoblots. Pattern I shows a polyclonal response in serum (S) and cerebrospinal fluid (CSF) (C) in a patient with nonspecific headache. Pattern II shows the same oligoclonal bands in serum and CSF (mirror pattern) in a patient with Guillaume-Barré syndrome. Pattern III shows oligoclonal IgG bands detected in serum, with more than 2 additional bands present in CSF, in a patient with viral encephalitis. Pattern IV shows more than 2 oligoclonal bands in CSF with a polyclonal distribution in the paired serum sample in a patient with multiple sclerosis.

TEST VALIDITY CALCULATION

The following ratios were used:

Sensitivity = [TP/(TP + FN)] × 100
Specificity = [TN/(TN + FP)] × 100
Positive predictive value = [TP/(TP + FP)] × 100
Negative predictive value = [TN/(TN + FN)] × 100
Accuracy = [(TP + TN)/(TP + TN + FP + FN)] × 100

STATISTICS

Results were analyzed using a statistical software package (Prism 3.0; GraphPad, San Diego, Calif). We used the Fisher exact test to compare percentages.

RESULTS

IgG PATTERNS

All results were interpreted by immunologists (L.M.V. and P.G.-P.) masked to diagnosis. Four different patterns were found in IgG immunoblots (Figure). In pattern I, a polyclonal response (no individual bands present) was detected in serum and CSF. In pattern II, the same OCBs were detected in serum and CSF (mirror pattern). In pattern III, OCBs were detected in serum and CSF, with more than 2 additional bands present in
CSF. In pattern IV, more than 2 OCGBs were detected in CSF with a polyclonal distribution in the paired serum sample. Patients with pattern III or IV were considered positive for ITGS; those with pattern I or II were considered negative for ITGS.

Intrathecal IgG synthesis was detected in 96.2% (127/132) of the patients with MS, 1.0% (1/100) with non-inflammatory neurologic diseases (a patient with an aggressive form of motor neuron disease), and 35.3% (18/51) with CNS infectious disorders. None of the 63 patients with other inflammatory neurologic diseases different from MS showed ITGS.

In the MS group, we did not find significant differences in the percentage of positive samples between patients who experienced lumbar puncture after their first relapse (88.9% [24/27]) and those in whom lumbar puncture was performed after a second attack, when the diagnosis of clinically definite MS was established (98.1% [103/105]). Consequently, all patients with MS were considered to be a unique group in subsequent studies.

Analysis of the patterns present in patients with ITGS showed that in CNS infections, pattern III was predominant (16 of 18 ITGS-positive samples). Conversely, pattern IV was mainly found in the MS group (104 of 127 ITGS-positive samples). In the patient with a non-inflammatory neurologic disease, pattern III was found.

The study of patients who lacked ITGS showed an increased percentage of mirror patterns in CNS infections (23 [45.1%] of 51 patients), neoplastic meningitis (6 [50%] of 12 patients), inflammatory diseases of the peripheral nervous system (15 [37.7%] of 35 patients), and inflammatory diseases of the CNS different from MS (16 [43.2%] of 37 patients). Table 1 summarizes the IgG patterns present in the different CNS inflammatory diseases studied. The mirror pattern is frequent in some of these diseases, such as Behçet disease, neurolupus, Rasmussen disease, and Hashimoto encephalitis. Conversely, this pattern is absent from the MS group.

A higher percentage of samples with pattern I (polyclonal) was detected in the control group (36 of 39 patients) and in patients with noninflammatory neurologic diseases (84 of 100 patients).

### Table 1. IgG Patterns in Patients With CNS Inflammatory Neurologic Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple sclerosis (n = 132)</td>
<td>5</td>
<td>23</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>Myelitis (n = 9)</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CNS vasculitis (n = 8)</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Paraneoplastic syndrome (n = 4)</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neurolupus (n = 4)</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Behçet disease (n = 3)</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rasmussen disease (n = 3)</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hashimoto encephalitis (n = 2)</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
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<td>Gluten ataxia (n = 2)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neurosarcoidosis (n = 1)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sjögren disease (n = 1)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviation: CNS, central nervous system.

*See the “Results” section for definitions of the IgG patterns.

### Table 2. Accuracy of Alkaline Phosphatase Immunodetection for the Diagnosis of Multiple Sclerosis*

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 385)</th>
<th>Group B (n = 334)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity, %</td>
<td>96.2</td>
<td>96.2</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>92.5</td>
<td>99.5</td>
</tr>
<tr>
<td>Accuracy, %</td>
<td>93.8</td>
<td>96.2</td>
</tr>
<tr>
<td>Positive predictive value, %</td>
<td>87.0</td>
<td>99.2</td>
</tr>
<tr>
<td>Negative predictive value, %</td>
<td>98.0</td>
<td>97.6</td>
</tr>
</tbody>
</table>

*Group A consists of the whole group of patients and group B consists of the whole group of patients excluding those with central nervous system infectious diseases.

### VALIDITY OF THE TEST FOR THE DIAGNOSIS OF MS

The results were analyzed considering patients with and without ITGS. The results of IEF were classified as true-positive (ITGS positive in patients with MS), true-negative (ITGS negative in patients without MS), false-positive (ITGS positive in patients without MS), and false-negative (ITGS negative in patients with MS). Sensitivity, specificity, accuracy, positive predictive value, and negative predictive value were calculated considering the results obtained in all patients (group A) and considering the results obtained excluding patients with CNS infections (group B). This distinction was made because most studies performed to evaluate sensitivity and specificity of OCGBs in the diagnosis of MS do not include CNS infections, considering that acute infectious diseases do not represent a differential diagnosis problem with MS. The sensitivity of the test was 96.2% in both groups. Specificity, accuracy, and negative predictive value were high in group A, and specificity, accuracy, and positive and negative predictive values were high in group B (Table 2).

### COMMENT

The clinical diagnosis of MS has been classically based on clinical criteria. Paraclinical tests are also used when necessary. When clinical criteria are exclusively applied at disease onset, a delay in the diagnosis is produced because disease evolution has to be followed up. Since the appearance of new therapies that modify the natural history of the disease, the search for methods that help establish an earlier diagnosis has become a subject of utmost importance. This search has principally been made in 2 different fields—magnetic resonance imaging and ITGS. Magnetic resonance imaging is useful for investigating lesion dissemination in time and space, thus contributing to an early diagnosis of MS. In addition, ITGS demonstrates an immunologic reaction in the CNS, improving the pathological specificity of magnetic resonance imaging findings. Oligoclonal band analysis is the more accurate method for detecting ITGS, and IEF followed by immunodetection has been postulated as the best method for detecting OCGBs. However, the methods widely used for OCGB detection require expert laboratories for the correct interpretation of a variety of cases.
causing variability of results among different laboratories. We recently developed a new method for OCBG detection that shows high sensitivity and definition and that is easier to interpret. Applying this technique, we exclusively found ITGS in MS, CNS infections, and 1 patient with motor neuron disease when 385 neurologic patients were studied. No patient with inflammatory neurologic diseases different from MS showed ITGS when assayed using our method. We found only polyclonal or mirror patterns in these patients. These results emphasize the importance of this new analysis in the differential diagnosis of MS from other inflammatory neurologic diseases.

Another interesting finding was observed in the 2 groups of patients with ITGS. Most patients with MS showed pattern IV (OCGBs restricted to CSF), and most patients with CNS infections showed pattern III (OCGBs present in serum, with additional bands in CSF). Thus, pattern IV detection is highly suggestive of MS. There were no statistically significant differences in sensitivity between the clinically definite and first relapse groups in MS. Thus, this technique can help in the early diagnosis of the disease.

The validity of this OCBG test for the diagnosis of MS was analyzed in 2 different ways. We analyzed the results obtained with the total group of patients and, in addition, excluding data from patients with CNS infections. We obtained a sensitivity of 96.2%, comparable with that previously described by expert laboratories for MS diagnosis, which ranges from 90% to 100%. However, the major advantage of this method lies in its specificity, which is even more important than sensitivity in a lifelong condition such as MS. Even including in the study the patients with CNS infections, where sometimes ITGS may be found, specificity was 92.5%, which is higher than that obtained with other methods, which ranges from 79% to 90.5%.

Although the usefulness of this technique in the diagnosis of MS should be confirmed with studies performed in multiple laboratories, these results reinforce the value of oligoclonal band studies in the early differential diagnosis of MS.

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Author Contributions: Study concept and design: Villar and Álvarez-Cermeño. Acquisition of data: Villar, Masjuan, Sádaba, Plaza, and Álvarez-Cermeño. Analysis and interpretation of data: Villar, Masjuan, González-Porqué, Bootello, and Álvarez-Cermeño.

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