Optimizing Detection of Oligoclonal Bands in Cerebrospinal Fluid by Use of Isoelectric Focusing With IgG Immunoblotting

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Multiple sclerosis (MS) is difficult to diagnose using clinical evidence alone, especially early in the course of the disease. Yet, the importance of early detection is increasing because immunomodulatory therapy is believed to be most beneficial early in the disease process.1

MS is first suspected when recurrent clinical symptoms point to local destruction of myelin in at least 2 anatomic locations within the central nervous system (CNS). Symptoms are highly variable, some with specific features such as diplopia, optic neuritis, paresthesias, numbness, poor vibration sensation, absence of abdominal reflexes, and trigeminal neuralgia.2 Other symptoms may be vague, such as weakness and fatigue. The nonspecific nature of symptoms makes early diagnosis difficult and clinical laboratory testing essential.

The anatomic lesions in the CNS are sites of demyelination and contain phagocytes and lymphocytes, including myelin-reactive T cells.3,4 Also within the CNS lesions are clones of plasma cells that produce a wide variety of antibodies. Some of these antibodies are directed against myelin and some against myelin oligodendrocyte glycoprotein, while many others have unknown specificity.5 Although the specificities of most of the immunoglobulins produced are not known, electrophoretic methods to detect the presence of these immunoglobulins have found a use in supporting the clinical diagnosis of MS.

As early as 1942, protein electrophoresis demonstrated that the cerebrospinal fluid (CSF) from patients with MS had an increased content of gamma globulin.6 However, because several other conditions share that finding, it was not until the development of high-resolution agarose gel electrophoretic techniques that the more specific finding of oligoclonal bands (O-bands) in the CSF was demonstrated to be of value in supporting the diagnosis of MS.7,8 Because the clones of plasma cells are located in the CNS, O-bands are present in the CSF but not in the serum from the vast majority of patients with MS.9 When the same O-bands are present in both serum and CSF, it is more likely that the patient has a systemic process rather than MS. Such a finding is considered not sufficiently specific to support the diagnosis of MS.10

Detection of O-bands in the gamma region is not specific for MS, even when these bands are absent from the serum. Neoplasia, cerebrovascular accidents, inflammation, and structural CNS lesions occasionally are associated with the presence of O-bands in the CSF. Yet, examination of the CSF for the presence of O-bands is still the best single laboratory test providing support for the diagnosis of MS because many of the non-MS conditions in which they appear can be distinguished clinically from MS. In the appropriate clinical setting, the presence of the O-bands provides powerful supportive evidence for the diagnosis.11

The first techniques to detect O-bands in the CSF used agarose gel electrophoresis. For these methods, at least 3 mL of CSF is required because the sample must be concentrated 40- to 80-fold to visualize the bands. Alternatively, some laboratories use silver stains to enhance the sensitivity, thereby eliminating the concentration step. Although this was an important advance from low-resolution techniques in which the bands were not visualized at all, several studies done since 1980 found agarose gel electrophoresis to be the least sensitive of available methods. Because the many commercial preparations available for agarose gel electrophoresis vary in their resolution of discrete bands, the studies on cases of clinically proven MS have reported sensitivities of from 45% to
77% using this technique.12-17 However, even with the techniques displaying the highest levels of resolution, a common problem with agarose gel electrophoresis is that bands often stain weakly and there are few of them.

With agarose gel electrophoresis techniques, a minimum of 2 bands are required for a positive result, with no bands present in the corresponding serum sample. The presence of only 1 band is not considered a positive result. Since agarose gel electrophoresis yields small bands from non-immunoglobulins such as γ trace protein and might contain artifacts that appear as O-bands, interpretation might vary between observers.18 These deficits in agarose gel electrophoresis are evident in the report by Fortini et al19 in this issue of the Journal. Their average positive specimen demonstrated only 2.2 O-bands. Furthermore, they noted a 5% to 10% disagreement in positive and negative results between the 2 individuals who interpreted the agarose gels.

The use of immunofixation as an improvement in the sensitivity of CSF electrophoresis was first suggested by Cawley et al20 in 1976, when they also reported that the protein bands seen in the CSF were composed of IgG. They pointed out that immunofixation can remove ambiguity by distinguishing between γ trace proteins and immunoglobulins. Immunofixation also is more sensitive and does not require concentration before electrophoresis, dramatically decreasing the required volume to as little as 10 µL of applied specimen (note that often up to 1 mL is needed to determine the IgG content because the CSF might require dilution for these sensitive methods). Cavuoti et al17 reported a sensitivity of 74% using immunofixation vs 57% using agarose gel electrophoresis in detecting CSF O-bands. More recently, using a commercially available semiautomated system to perform immunofixation on CSF, Richard et al21 reported a sensitivity of 83% with a specificity of 79% for patients with clinically definite MS. Whereas the use of immunofixation is an improvement over agarose gel electrophoresis, immunofixation provides fewer bands than isoelectric focusing and IgG immunoblotting (IgG-IEF), and the bands by immunofixation tend to be diffuse.

In 1994, a consensus report from the Committee of the European Concerted Action for MS concluded that IgG-IEF is the most sensitive method for detection of CSF O-bands.22 This report has been confirmed by studies directly comparing the same cases by IgG-IEF and a variety of agarose gel electrophoresis techniques.11 For example, Lunding et al12 detected all 20 definite cases of MS by IgG-IEF, but only 9 of those 20 were positive by agarose, and Seres et al13 detected 91% of 37 cases of MS by IgG-IEF, but only 68% with agarose.

Similarly, the semiautomated IgG-IEF technique reported by Fortini et al19 in this issue of the Journal had a 90% sensitivity vs 60% for agarose. The specificities of these 2 methods are comparable: 94% for IgG-IEF and 96% for agarose.19 Perhaps the most telling feature is the reproducibility of the results with the IgG-IEF technique. Because the average positive case contained only 2.2 O-bands (with a requirement of 2 bands for a positive test result) for agarose gel electrophoresis, the difference between a positive and a negative report might be the subjective interpretation of 1 faintly staining band. However, for IgG-IEF in which an average of 8.0 bands was present in a positive case (with a requirement of 4 bands for a positive test result), the interpretation is far less likely to hinge on such subjective features. This provides an improvement in the confidence of a positive result with the IgG-IEF technique over that from agarose gel electrophoresis. Even more impressive is the finding that 17 of the 20 cases of definite MS reported had more than 8 bands. This likely explains why no discrepancies in positive and negative interpretations were found between the 2 observers when using the IgG-IEF technique.19

According to the 2002 College of American Pathologists Survey M-B, more than 90% of the 235 laboratories performing analysis of CSF for the detection of oligoclonal bands reported using electrophoresis, with fewer than 10% using IEF.23 The demonstration of superior sensitivity using an available commercial method for IgG-IEF described in this issue of the Journal will likely change these numbers.

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References


