

**Article abstract**—Although the ultimate diagnosis of multiple sclerosis (MS) still remains clinical, confirmatory laboratory aids can be of marked assistance, especially in early and atypical cases. Whereas numerous tests have been described, the only ones to withstand scrutiny in numerous laboratories have been various immunoglobulin assays and myelin basic protein measurements in the cerebrospinal fluid (CSF). Of the newer assays that are commercially available and readily adapted to routine clinical laboratory use, the most discriminating are the production of a CSF IgG:albumin ratio, using an electroimmunodiffusion method, and agarose electrophoresis of concentrated CSF to demonstrate oligoclonal IgG bands. Together, these assays can be performed on less than 3 ml of CSF, and will show relatively specific abnormalities in over 95% of clinically definite MS patients. They both detect abnormalities that frequently occur in the course of disease, and thus add considerable weight to the clinical impression of MS.

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# Cerebrospinal fluid and blood assays of diagnostic usefulness in multiple sclerosis

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The diagnosis of multiple sclerosis (MS) is frequently difficult, and some authorities indicate that it can be made with certainty only at autopsy.<sup>1</sup> Not surprisingly, numerous attempts have been made to develop a sensitive and reliable diagnostic test, and many claims of such a feat have been published, some of which are quite extravagant. Although many of these tests have failed to achieve wide acceptance, some may be of diagnostic value with further evaluation. An example of delayed acceptance of a useful procedure is agarose electrophoresis of cerebrospinal fluid (CSF) (see below), which has been widely used in Europe for over a decade but is just now receiving acceptance in the United States. Nevertheless, several consistent abnormalities have been detected in MS body fluids. These have been confirmed repeatedly in numerous laboratories and can now provide useful back-up to the clinical diagnosis. Several recent reviews address this subject, of which the ones by Tourtellotte,<sup>2</sup> Thompson,<sup>3</sup> and Johnson and Nelson<sup>4</sup> provide a comprehensive overview. This paper will consider spinal fluid abnormalities

first, and then consider recently described alterations in blood. The CSF changes occur within the immunoglobulins (quantitative, qualitative, and antibody abnormalities) or in the abnormal appearance of myelin basic protein (MBP) and its fragments.

**Cerebrospinal fluid assays.** Several widely available CSF measurements indicate abnormalities often noted in MS patients. Modest increases in the number of leukocytes per cubic millimeter are often observed; however, less than one-third of patients show this abnormality at any one time, and the number of cells is rarely greater than 50. Between one-third and one-half of patients with apparently active disease will also show mild increases in total protein content of CSF. The most widely used diagnostic indicator presently available is a relative increase in the gamma globulin or immunoglobulin (IgG) component of proteins, which is usually expressed as a percentage of total CSF protein. Approximately two-thirds of patients studied in large series show

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increases above the normal level of 11, although the percentage rarely exceeds 20. Several routine methods of measuring gamma or immunoglobulins are available, and they have been compared by Ansari, Wells, and Vatassery.<sup>5</sup> Routine electrophoresis, electroimmunodiffusion, and radial immunodiffusion methods were found to be roughly comparable in a series of 44 MS patients and closely matched controls. Of the three methods, the most sensitive and most easily performed was radial immunodiffusion, which was recommended as the method of choice for most clinical laboratories.

Several investigators have attempted to improve sensitivity by measuring both IgG and albumin to develop an IgG:albumin ratio in CSF or, in some studies, an index for measuring IgG:albumin ratios in both CSF and serum. Such studies are of considerable value, for they indicate that the IgG is frequently elevated out of proportion to the albumin in the CSF, thus strongly suggesting that IgG is synthesized within the central nervous system (CNS) in the majority of MS patients.<sup>9</sup> In several large series, such studies provide evidence of CSF abnormalities in 90% of MS spinal fluids. The electroimmunodiffusion method described under various names both by Tourtellotte et al<sup>6</sup> and Laurell<sup>7</sup> allows easy measurement of both IgG and albumin in a single antibody-impregnated plate. Reagents for this test are commercially available in the United States (Antibodies, Inc., Davis, CA), and it has found acceptance as a diagnostic MS test. Analyses of ratios between IgG and other specific CSF proteins have been less widely accepted, and measurements of single proteins other than IgG groups have not been useful in the diagnosis of MS.

Diagnostically useful qualitative abnormalities in MS CSF immunoglobulins have been recognized by numerous European investigators including Lowenthal, van Sande, and Karcher,<sup>8</sup> Laterre et al,<sup>9</sup> Link and Muller,<sup>10</sup> and Vandvik and Skrede<sup>11</sup> for some time. Using either agar or, more recently, agarose gels and concentrated CSF for high-resolution electrophoresis, all these authors have studied an extremely interesting migration pattern of IgG in which antibody populations segregate into discrete bands known as oligoclonal IgG bands. This type of abnormality is evident in between 85 and 95% of CSFs obtained from clinically definite MS patients.

The pattern is not unique to MS, however, and is seen in similarly treated CSF from most cases of chronic CNS infection: neurosyphilis, subacute sclerosing panencephalitis, progressive rubella panencephalitis, and many cases of chronic fungal meningitis. Oligoclonal IgG bands also occur in CSF in the persistent CNS viral infection of sheep visna, and have been noted in human CNS granulomatous angiitis (K. P. Johnson; unpub-

lished data). The demonstration of this abnormality in the vast majority of MS patients and in a large percentage of patients with chronic CNS infection has, of course, stimulated intense interest in such bands as possible clues to the etiology of MS. Johnson et al<sup>12</sup> have recently described a simple, commercially available method (Panagel, Worthington Laboratories, Freehold, NJ) for defining oligoclonal IgG bands in CSF, which is suitable for the routine clinical laboratory. It is highly sensitive and shows distinct abnormalities in approximately 90% of clinically definite MS patients. For those wishing more control of the test, the technique for agarose electrophoresis is relatively simple, and most clinical laboratories can probably produce high-quality gels without difficulty. Commercial protein concentrators are likewise available and easy to use, allowing rapid 50 to 85 × concentration of CSF so that the protein content is approximately that of serum.

Several laboratories have reported successful application of isoelectric focusing techniques of concentrated CSF proteins in both polyacrylamide and agarose gels.<sup>13</sup> Abnormal populations of IgG can be separated by this technique, and the bands are usually extremely alkaline, migrating further toward the cathode than most normal CSF immunoglobulins. The number of patients showing this abnormality is approximately the same as with agarose electrophoresis; however, there are fewer atypical or dubious protein banding patterns with isoelectric focusing techniques. The degree of difficulty and sensitivity of this assay is such that it probably will not replace agarose electrophoresis as a basic diagnostic test of MS, but it may be quite useful when an unequivocal answer cannot be obtained by the other technique. Commercially prepared gels and apparatus for performing isoelectric focusing of CSF are available.<sup>14</sup>

The combined use of sensitive quantitative techniques such as electroimmunodiffusion and qualitative techniques like agarose electrophoresis of concentrated CSF will confidently show distinct abnormalities in over 95% of clinically definite MS patients.<sup>4</sup> Such abnormalities are frequently present very early in the disease; thus, in incipient and atypical cases, they are of major benefit. Such tests have been highly useful in sorting out those cases of MS characterized as chronic progressive myelitis, which occasionally appear in somewhat older populations.<sup>15</sup>

During the last 15 years, it has been repeatedly shown that titers of viral antibodies are frequently elevated in MS CSF.<sup>16</sup> Although the first antibodies to be detected were to measles, and although most studies detect elevations of measles antibodies in a larger proportion of patients than with any other viral antibody, it is possible to detect elevated titers to rubella, vaccinia, and, at times, varicella zoster as well.<sup>17</sup> This finding has

excited much interest and study without, unfortunately, leading to a clear-cut understanding of why such antibodies are there. These studies have thus far failed to provide a strong link between a past or persistent viral infection and MS. It was hoped that the measurement of viral antibodies would be of diagnostic usefulness in MS; however, this has not been the case. In an extensive, recently completed serologic study of MS CSF and serum, we found that antibodies to several viruses were present in increased titer in MS CSF compared with the CSF of matched controls.<sup>18</sup> Nevertheless, when all the viral antibody abnormalities were combined, they failed to segregate the MS CSF from control CSF as well as from the presence of oligoclonal IgG bands.

After the observation by Herndon and Johnson<sup>19</sup> of myelin fragments in the CSF of MS patients during acute attacks, a search has been made for more quantitative methods of detecting MBP in MS CSF. Several investigators<sup>20-22</sup> have succeeded in adapting radioimmunoassays to this task. Such assays are now relatively well standardized and are capable of detecting minute amounts of MBP fragments. The assays require highly specific antisera and have not been developed to the point of widespread use in the clinical laboratory. Nevertheless, they show promise of clinical usefulness in determining recent demyelination or acute new disease activity in the MS patient. They have not been used as a basic diagnostic test for the disease, however.

**Blood.** All the preceding assays have required CSF, and a continuing search is being made for a valid blood test that would allow the diagnosis of MS to be made without lumbar puncture. Levy, Auerbach, and Hayes<sup>23</sup> have described a rosetting test in which the increased adherence of peripheral blood lymphocytes to measles virus-infected cells was found to be more common in MS patients than in controls. Since the original report, others have been unable to confirm the findings or have found that the test is quite difficult and not suitable for routine diagnostic application.<sup>24,25</sup> An explanation for these conflicting results may possibly be found in recent work showing that normal lymphocytes treated with prostaglandin E<sub>1</sub> showed increased ability to form rosettes with measles-infected cells,<sup>26</sup> whereas treatment of MS patients with aspirin, which inhibits prostaglandin effects, reduces their ability to form rosettes.<sup>27</sup> Although this interesting series of experiments shows continued promise, the use of peripheral blood lymphocytes in rosetting tests has not yet been standardized for clinical application.

Quite recently, Angers et al<sup>28</sup> described a leukocyte adherence inhibition assay as a possible diagnostic test for MS. This assay "measures a decrease in the ability of leukocytes to adhere to a glass

surface when exposed to antigens to which the leukocytes have been sensitized." The test is specific for MS according to the initial report; however, larger numbers of controls with various neurologic diseases must be tested, and confirmation by independent laboratories will be necessary before it can be evaluated adequately.

## References

1. McDonald WI: What is multiple sclerosis? Clinical criteria for diagnosis. In Davison AN, Humphrey JH, Liversedge AL, et al (Editors): *Multiple Sclerosis Research*, Amsterdam-New York, Elsevier Scientific Publishing Co, 1975, p 1
2. Tourtellotte WW: What is multiple sclerosis? Laboratory criteria for diagnosis. In Davison AN, Humphrey JH, Liversedge AL, et al (Editors): *Multiple Sclerosis Research*. Amsterdam-New York, Elsevier Scientific Publishing Co, 1975, p 9
3. Thompson EJ: Laboratory diagnosis of multiple sclerosis: Immunological and biochemical aspects. *Br Med Bull* 33:29-33, 1977
4. Johnson KP, Nelson BJ: Multiple sclerosis: Diagnostic usefulness of cerebrospinal fluid. *Ann Neurol* 2:425-431, 1977
5. Ansari KA, Wells K, Vatassery GT: Quantitative estimation of cerebrospinal fluid globulins in multiple sclerosis: A comparison of electrophoretic and immunologic methods. *Neurology (Minneapolis)* 25:688-692, 1975
6. Tourtellotte WW, Tavolato B, Parker JA, et al: Cerebrospinal fluid electroimmunodiffusion: An easy, rapid, sensitive, reliable, and valid method for the simultaneous determination of immunoglobulin-G and albumin. *Arch Neurol* 25:345-350, 1971
7. Laurell CS: Electroimmuno assay. *Scand J Clin Lab Invest* 29 [Suppl 124]:21, 1972
8. Lowenthal A, van Sande M, Karcher D: The differential diagnosis of neurological diseases by fractionating electrophoretically the CSF G-globulins. *J Neurochem* 6:51-56, 1960
9. Laterre EC, Callewaert A, Heremans JF, et al: Electrophoretic morphology of gamma globulins in cerebrospinal fluid of multiple sclerosis and other diseases of the nervous system. *Neurology (Minneapolis)* 20:982-990, 1970
10. Link H, Muller R: Immunoglobulins in multiple sclerosis and infections of the nervous system. *Arch Neurol* 25:326-344, 1971
11. Vandvik B, Skrede S: Electrophoretic examination of cerebrospinal fluid proteins in multiple sclerosis and other neurological diseases. *Eur Neurol* 9:224-241, 1973
12. Johnson KP, Arrigo SC, Nelson BJ, et al: Agarose electrophoresis of cerebrospinal fluid in multiple sclerosis: A simplified method for demonstrating cerebrospinal fluid oligoclonal immunoglobulin bands. *Neurology (Minneapolis)* 27:273-277, 1977
13. Laurenzi MA, Link H: Characterization of the mobility on isoelectric focusing of individual proteins in CSF and serum by immunofixation. *J Neurol Neurosurg Psychiatry* 42:368-372, 1979
14. Hosein Z, Johnson KP: Isoelectric focusing of cerebrospinal fluid (CSF) proteins for the diagnosis of multiple sclerosis. *Clin Chem* 25:1131, 1979
15. Link H, Norrby E, Olsson J-E: Immunoglobulin abnormalities and measles antibody response in chronic myelopathy. *Arch Neurol* 33:26-32, 1976
16. Norrby E: Viral antibodies in multiple sclerosis. *Prog Med Virol* 24:1-39, 1978
17. Forghani B, Cremer NE, Johnson KP, et al: Viral an-

tibodies in cerebrospinal fluid of multiple sclerosis and control patients: Comparison between radioimmunoassay and conventional techniques. *J Clin Microbiol* 7:63-69, 1978

18. Johnson KP, Cremer NE, Forghani B: Viral antibody and protein studies of cerebrospinal fluid: Diagnostic usefulness in multiple sclerosis. In Lowenthal A (Editor): *Humoral Immunity in Neurological Disease*. New York, Plenum Press, 1979, pp 233-237
19. Herndon RM, Johnson RT: A method for the electron microscopic study of cerebrospinal fluid sediment. *J Neuropathol Exp Neurol* 29:320-330, 1970
20. Hsiung HM, Wu J, McPherson TA: Silica gel radioimmunoassay for myelin basic protein. *Clin Biochem* 11:54-56, 1978
21. Cohen SR, Herndon RM, McKhann GM: Myelin basic protein in cerebrospinal fluid as an indicator of active demyelination. *Arch Neurol* 33:384, 1976
22. Whitaker JN: Myelin encephalitogenic protein fragments in cerebrospinal fluid of persons with multiple sclerosis. *Neurology (Minneapolis)* 27:911-920, 1977

23. Levy NL, Auerbach PS, Hayes EC: A blood test for multiple sclerosis based on the adherence of lymphocytes to measles-infected cells. *N Engl J Med* 297:1423-1427, 1977
24. Salmi A, Fray H: More about blood test for MS. *N Engl J Med* 297:398-399, 1977
25. Dore-Duffy P, Zurier RB, Donaldson J, et al: Lymphocyte adherence in multiple sclerosis. *Neurology* 29:232-235, 1979
26. Zurier RB, Dore-Duffy P, Viola MV: Adherence of human peripheral lymphocytes to measles-infected cells: Enhancement of prostaglandin E<sub>1</sub>. *N Engl J Med* 296:1143-1146, 1977
27. Dore-Duffy P, Zurier RB: Lymphocyte adherence in multiple sclerosis: Effect of aspirin. *J Clin Invest* 63:154-157, 1979
28. Angers JW, Korik L, Franklin LS, et al: The leukocyte adherence-inhibition assay as a diagnostic test for multiple sclerosis: Study of a substance found in MS blood. *J Lab Clin Med* 93:528-534, 1979