Oligoclonal Bands in Cerebrospinal Fluids: Significance of Corresponding Bands in Serum for Diagnosis of Multiple Sclerosis

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Controversy exists regarding the definition of oligoclonal bands in cerebrospinal fluid (CSF) and whether CSF bands with corresponding bands in the serum should be disregarded in the interpretation. Much of this controversy results from not distinguishing between the sensitivities of different techniques used for these studies. Because the combination of isoelectric focusing/silver staining is more sensitive than agarose gel electrophoresis/Coomassie Blue staining for detecting weak bands, one would expect to find weak oligoclonal bands in the serum more frequently with the former technique than the latter. Yet, most clinical laboratories use agarose gel electrophoresis for this assay. To investigate the clinical relevance of CSF oligoclonal bands as compared with corresponding bands for serum by electrophoresis on agarose gel, we retrospectively reviewed paired CSF and serum electrophoretograms of 104 patients. We determined that the specificity of the oligoclonal-band test for diagnosis of multiple sclerosis was significantly enhanced by running paired CSF and serum specimens when CSF bands with corresponding serum bands were discounted.

The presence of oligoclonal bands (OB) in the gamma region of electrophoretograms of cerebrospinal fluid (CSF) is well known to be supportive evidence of multiple sclerosis (MS). However, there is controversy as to the best definition of OB. Some authors have used paired serum and CSF specimens and tended to disregard, as nonspecific, those bands with identical migration in serum and CSF (1). Others believe that this may exclude cases of MS that can have OB in the serum (2). This controversy reflects differences in some of the techniques used to detect OB.

To evaluate the usefulness of running paired specimens by high-resolution agarose gel electrophoresis in the routine clinical laboratory, we have reviewed the electrophoreograms of 54 patients with bands in the gamma region of CSF and of 50 patients with no bands. A positive result for oligoclonal bands was defined as the presence of more than one band in the gamma region of CSF without corresponding bands in the serum. To establish the diagnosis, we reviewed the clinical presentation without reference to laboratory findings. We found that comparison of serum and CSF for OB significantly improved the specificity for the diagnosis of MS without significantly decreasing the sensitivity. A possible reason for these findings is discussed.

Materials and Methods

All CSF electrophoreses performed in the Clinical Immunology Laboratory at the University of Michigan during 1986 were reviewed. All specimens with at least one band in the gamma region that also had an accompanying serum specimen were selected (54 patients). A similar number of specimens from age- and sex-matched patients in which no bands were seen in the gamma region were also selected (50 patients).

For electrophoresis in agarose gel we used the Panigel System (Grafar, Detroit, MI), running CSF and a serum sample from the same patient in parallel. CSF specimens (2 mL) were concentrated 80-fold in Minicon concentrators (Amicon, Danvers, MA) before application to the gel. Serum specimens were diluted fourfold. Electrophoresis was for 35 to 40 min at 200 V. After fixation in picric acid, gels were stained with Coomassie Blue.

A specimen was considered positive for OB if we saw in the gamma region of CSF two or more bands, other than gamma trace protein, for which there were no corresponding bands in the serum. Medical records were reviewed to determine the clinical diagnosis. A diagnosis of multiple sclerosis was established by clinical criteria of Rose et al. (3) without reference to the presence or absence of oligoclonal bands. For the purposes of this study, cases of definite, probable, and possible multiple sclerosis were categorized as "MS." These patients had a relapsing and remitting, or slowly progressive, course with neurologic signs referable to more than one site of central nervous system white matter, with no better neurologic explanation.

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1 Nonstandard abbreviations: CSF, cerebrospinal fluid; MS, multiple sclerosis; OB, oligoclonal bands.

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Results

When positive OB are defined by our criteria, 28 patients with MS were positive and five were negative. Fifteen patients without MS were positive and 56 were negative. None of the five negative patients had above-normal values for CSF total protein, CSF IgG index, or CSF myelin basic protein. Fifteen patients without MS had positive OB: four with encephalitis, three with dementia, and one each with acute lymphocytic leukemia (in remission, receiving intrathecal chemotherapy), cryptococcal meningitis, systemic lupus erythematosus with involvement of the central nervous system, paraneoplastic syndrome (breast carcinoma), cervical myelopathy, recent stroke, neurosyphillis, or functional gait disorder. All but four of these patients (one with a functional gait disorder, one with cervical myelopathy, and two with dementia) had clinical or laboratory evidence of a systemic or central nervous system inflammatory process. These results are highly statistically significant (chi square test, \( P < 10^{-8} \)). The sensitivity for the diagnosis of MS was 84.9%, specificity 78.9%.

Figure 1a illustrates a positive OB gel with three bands in CSF and one band in serum. This patient had clinical MS. Figure 1b illustrates a negative OB gel with four bands in CSF and three in serum. This patient had dementia.

If the serum results are disregarded and positive OB are defined as more than one band in the gamma region of CSF other than gamma trace protein, then 29 patients with MS were positive and four were negative. However, 25 patients without MS were also positive and 46 were negative. These results are still statistically significant (chi square test, \( P = 1.4 \times 10^{-5} \)). The sensitivity increased slightly to 87.9%, but the specificity decreased to 64.8.

Discussion

The presence of OB in the gamma region of CSF is supportive evidence for the diagnosis of MS. However, it is well known that various other pathological conditions can result in positive OB (5). The bands are thought to represent the intrathecal synthesis of immunoglobulins, which do not diffuse across the blood–brain barrier to any great extent. Detection of OB can be enhanced by using isoelectric focusing, a more sensitive technique, but the number of false positives will also increase (4). Thus, this increased sensitivity is a very mixed blessing: although one can better detect weak bands in the electrophoretograms for serum of these patients, this information serves to obfuscate the interpretation, because the bands cannot be easily quantified.

The present study demonstrates that high-resolution electrophoresis of CSF is sufficiently sensitive for the detection of oligoclonal bands in the vast majority of MS patients, at a similar sensitivity as has been observed by others (4, 5). Nonetheless, some have argued that running a parallel serum sample and defining positivity as we have may increase the number of false-negative results (2). When isoelectric focusing is used, corresponding bands in CSF and serum have been demonstrated in as many as 45% of MS patients (6). However, in the same study, 9% of MS patients had parallel bands in serum and CSF on agarose gel electrophoresis, the technique used being similar to ours. This observation would indicate that the serum bands seen by the more-sensitive technique may indeed reflect intrathecal synthesis of immunoglobulin that has entered the bloodstream, whereas serum bands seen by routine agarose gel electrophoresis are more likely to indicate systemic synthesis of immunoglobulin that has crossed the blood–brain barrier into the CSF. This study demonstrates a significant improvement in specificity when a parallel serum sample is run and positive OB are defined according to our criteria.

The presence of OB in CSF may also be supportive evidence for an intrathecal inflammatory process other than MS. In this study, 15 such patients had positive oligoclonal bands, and 11 of them had clinical or laboratory evidence of an inflammatory process.

We conclude that high-resolution agarose gel electrophoresis of CSF, stained with Coomassie Blue, is a sensitive test for OB and that when a simultaneous serum sample is run and positive OB are defined as two or more bands in the gamma region of CSF without corresponding bands in the serum, the specificity is significantly increased.

References