

Recommended Standard of Cerebrospinal Fluid Analysis in the Diagnosis of Multiple Sclerosis

A Consensus Statement

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New criteria for the diagnosis of multiple sclerosis (MS) were published as the result of an internationally formed committee. To increase the specificity of diagnosis and to minimize the number of false diagnoses, the committee recommended the use of both clinical and paraclinical criteria, the latter involving information obtained from magnetic resonance imaging, evoked potentials, and cerebrospinal fluid (CSF) analysis. Although rigorous magnetic resonance imaging requirements were provided, the “new criteria paper” fell short in terms of guidelines as to how the CSF analysis should be performed and simply equated the IgG index with isoelectric focusing, without any justification. The spectrum of parameters analyzed and methods for CSF analysis differ worldwide and often yield variable results in terms of sensitivity, specificity, accuracy, and reliability, with no decided “optimal” CSF test for the diagnosis of MS. To address this question specifically, an international panel of experts in MS and CSF diagnostic techniques was convened and the result was this article, representing a consensus of all the participants. These recommendations for establishing a standard for the evaluation of CSF in patients suspected of having MS should greatly complement the new criteria in ensuring that a correct diagnosis of MS is being made.

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An incorrect diagnosis of multiple sclerosis (MS) causes great consternation to patients and may lead to unnecessary treatment with expensive agents. To minimize this risk and maintain a high level of disease specificity, a set of new recommended diagnostic criteria were recently published¹ that use both clinical and paraclinical information in an algorithm that allows for only 3 categories of diagnostic

certainty: MS, possible MS, and not MS. The criteria emphasize a clinical diagnosis, relegating any paraclinical measure (ie, magnetic resonance imaging [MRI], evoked potentials, or cerebrospinal fluid [CSF]) to being only supportive and not itself diagnostic. The criteria use one paraclinical test to improve the specificity of another paraclinical test. For instance, only 2 MRI-detected lesions consistent with MS in the presence of oligoclonal bands or a raised IgG index are sufficient to fulfill the new criteria’s definition for “dissemination in space” whereas such an MRI result by itself is not.¹ To maximize the benefit of CSF as a diagnostic paraclinical test, the most sensitive method should be used. The power of paraclinical tests may be even greater when some doubt is cast in clinical diagnosis. When the results of the paraclinical tests are normal, this strongly suggests an alternative diagnosis; whereas

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when they are abnormal, they would support the primary diagnosis. In addition, in line with the first new criterion of "no better explanation" other than MS to account for the historical and objective evidence of neurological dysfunction, the use of paraclinical tests help to rule out other conditions. Cerebrospinal fluid analysis plays an important role in this regard, but the new criteria fall short in recommending how this analysis should be carried out. There are many different techniques to evaluate CSF and no consistent standard is used among the laboratories in either the testing or reporting of CSF results. Some techniques claim to offer greater sensitivity and specificity regarding the qualitative or quantitative abnormalities that are being measured in support of a diagnosis of MS. In an effort to evaluate and recommend the type of CSF analysis that yields the greatest specificity, in line with the main objective of the new criteria, the Consortium of Multiple Sclerosis Clinics commissioned a study group that included individuals who had considerable expertise in diagnosing and managing patients with MS, CSF analysis, or both. Its mandate was to produce a report for neurologists and laboratory medicine specialists that detailed what would be considered the "minimum standard" for evaluation of CSF in patients suspected of having MS. Based on all the information presented and reviewed, this is the report of that study group.

THE CSF REPORT

All aspects of CSF analysis will help distinguish between other causes of systemic inflammation that spill over into the central nervous system and might mimic MS, such as vasculitis or chronic infection. A white blood cell (WBC) count and differential cell count as well as protein concentration and glucose level help to round out the CSF picture together with the more specific albumin and immunoglobulin measurements. The cell count should be performed no later than 2 hours after obtaining the CSF, otherwise changes in cell shape may hamper the ability to offer a correct and full differential cell count. A red blood cell count that is too high ($5 \times 10^9/L$ to $7 \times 10^9/L$) probably indicates a traumatic tap, rendering other quantitative measurements possibly uninterpretable. Higher than normal [N] ($<5 \times 10^6/L$) WBC counts are found in some 34% of MS cases,² very high CSF WBC counts ($>50 \times 10^6/L$) are unusual in MS. Low CSF glucose levels (when compared with serum, CSF/serum ratio <0.4) and very high total protein content (eg, >1 g/L) are more consistent with an infectious or neoplastic process. Lactate, where available, is a good substitute and has an advantage over paired CSF-plasma glucose measurements in that only a single CSF measurement is required.³ The age-related evaluation of the CSF-serum albumin quotient is preferred for its higher accuracy than total protein level to detect a blood-CSF barrier (BCB) dysfunction. The albumin quotient is also the basis for the different concepts of quantitation of the intrathecal immunoglobulin response. A complete CSF data report is the standard for many laboratories in Europe and in some laboratories in North and South America and the Middle East.^{4,5}

METHODS OF IMMUNOGLOBULIN ANALYSIS

The Sample

Cerebrospinal fluid should be studied neat or unadulterated; concentrating CSF prior to performing analyses is obsolete because most immunoglobulin studies can be performed using only a small volume of CSF (<1 mL). For qualitative immunoglobulin analyses⁶ some laboratories load a known amount equally from serum and CSF (eg, 40 ng), whereas others use a fixed dilution of serum (eg, 1:400) and run it against a set volume (eg, 4 μ L) of CSF. If gels are overloaded with protein or if insufficient immunoglobulin is loaded, then the interpretation is difficult and requires the samples be rerun. For immunoblotting of immunoglobulin, samples should contain anywhere from 20 to 1200 ng of IgG, translating in most cases into 3 to 5 μ L of CSF. Using this quantity of unconcentrated CSF, rarely has there been a problem with underloading or overloading of gels in experienced laboratories.

Basic Program of CSF Analysis for Diagnosis of Neurological Diseases

Neurologists need to consider the results of *all* of the other tests performed as part of the CSF panel (eg, cell count; protein, glucose, and lactate levels; and others). There are many different techniques to measure the amount of immunoglobulin present within the CSF and serum sample to determine whether the immunoglobulin was synthesized locally within the CSF or had diffused in a normal or abnormal BCB. (The term *blood-CSF barrier* is recommended over blood-brain barrier in this context.) Published formulas help to accurately assess the integrity of the BCB. Hyperbolic and exponential functions describing CSF IgG synthesis are clinically equivalent and both are more accurate than the simplified IgG index.⁷⁻¹⁰

Some improved sensitivity has been reported with modifications to this formula.¹¹ There may be a complementary role for quantitative IgG measurement in the diagnosis of MS. With no better explanation, most patients with a raised IgG index will have MS, but sensitivities vary and specificities fall, especially owing to the increase in false-positive results if a hyperbolic function is not used. However, results from other laboratories¹² suggest that even with hyperbolic function correction, quantitative IgG analysis will generally pick up only around 75% of the patients who will turn out to be oligoclonal band positive.

QUALITATIVE VS QUANTITATIVE DETECTION OF INTRATHECAL IGG IN MS

Regarding the lower sensitivity of quantitative vs qualitative analysis in the detection of intrathecal IgG synthesis in MS, this derives from the fundamental differences that the 2 techniques use to distinguish normal from abnormal. In quantitative analysis, each patient is compared with a large population and, hence, wide reference range of blood-derived proteins in CSF whereas in qualitative analysis each patient's CSF IgG pattern is compared with his or her own parallel serum sample.

Therefore, our consensus for the diagnosis of MS is that the IgG index or any other quantitative IgG analysis is not equivalent to qualitative analysis using isoelectric focusing with immunofixation, as opposed to the previous recommendation that equated the IgG index with qualitative analysis.¹

Table 1. Improved Specificity of Immunoblotting vs Silver Staining*

Result	PAGE/IEF With Silver Staining	IEF With Immunoblotting
True positive	14	33
False positive	12	2

Abbreviations: IEF, isoelectric focusing; PAGE, polyacrylamide gel electrophoresis.

* $P < .02$ by χ^2 test.

Qualitative Methods in CSF Analysis

There is complete agreement that isoelectric focusing (IEF) on agarose gels followed by immunoblotting¹³ should be the "gold standard" for detecting the presence of oligoclonal bands. Other methods such as polyacrylamide gel combined with IEF and silver staining of proteins might have proven useful in the past, but they lack specificity for IgG and, hence, are not supported by consensus.^{14,15} A direct comparison of the accuracy of the 2 techniques is given in **Table 1**.²⁰ Compared with IEF and IgG immunoblotting, direct silver staining techniques demonstrate reduced sensitivity and specificity in diagnosing MS. Examples of the preferred method for evaluation of oligoclonal bands with IEF and immunoblotting are shown in the **Figure**.

Pattern type 1 is considered negative (ie, no specific CSF bands), whereas pattern type 2 definitively shows specific bands present only in the CSF but not the serum sample. Pattern types 3 and 4 require more careful interpretation. In particular, type 4 can be misinterpreted if the amount of IgG in the serum sample is too high, which can blur the serum bands. This is one reason for adding equal amounts of IgG from the CSF and the serum sample. Type 4 can be seen in conditions such as the Guillain-Barré syndrome. Pattern type 5 indicates the presence of a monoclonal gammopathy, but IEF resolves what would be a single band using other electrophoretic techniques into multiple bands differing by 1 U of charge. This peculiarity is probably due to posttranslational modifications such as glycosylation.

The method for this technique of IEF and immunoblotting has been standardized and is commercially available. Using this technique requires a certain level of technical expertise and the interpretation similarly necessitates some experience. This is best left to laboratories and clinical biochemists with experience in CSF diagnostics.²¹ Each gel run requires the presence of certain controls that help to determine the reliability of any given run. Knowing when a run is "interpretable" is where the expertise is most needed.

Some laboratories also stain for κ and/or λ light chains (both free and bound) since a given IgG can only be associated with one or the other.²² IgG bands are discerned against a polyclonal background; light chain staining reduces this polyclonal background substantially so that faint bands are better seen. If a single specific band is seen in the CSF, or only faint bands are seen, but bands resolve with staining for light chains, then this would imply there are, in fact, oligoclonal bands that fail to resolve on IgG staining. Light chain staining would also be positive in rare cases where oligoclonal bands are caused by the presence of IgA or IgM, which will not appear on gels stained only for IgG. Some laboratories have proceeded with this analysis in patients in whom MS is strongly suspected on the basis of their clinical or MRI findings but were negative on IgG oligoclonal band testing.²³ Most of these laboratories analyze the presence of free, as opposed to bound, light chains, which are often synthesized in excess of immunoglobulin heavy chains by plasma cells. Any staining for light chains in the CSF is most likely caused by local synthesis since

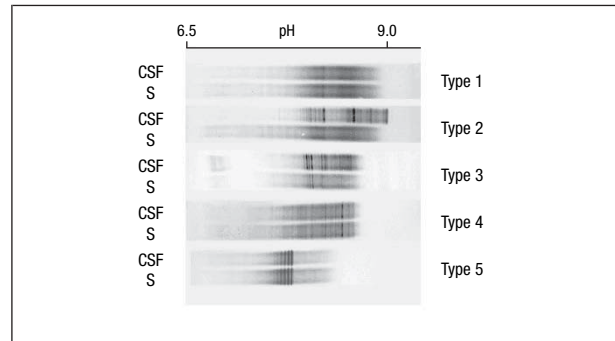


Figure. Isoelectric focusing on agarose gels with immunoblotting. Note that all the oligoclonal bands present are due to IgG. There are 5 classic patterns: type 1, no bands in cerebrospinal fluid (CSF) and serum (S) sample; type 2, oligoclonal IgG bands in CSF, not in the S sample, indicative of intrathecal IgG synthesis; type 3, oligoclonal bands in CSF (like type 2) and additional identical oligoclonal bands in CSF and the S sample (like type 4), still indicative of intrathecal IgG synthesis; type 4, identical oligoclonal bands in CSF and the S sample illustrative of a systemic not intrathecal immune reaction, with a leaky or normal or abnormal blood-CSF barrier and oligoclonal bands passively transferred in the CSF; and type 5, monoclonal bands in CSF and the S sample; this is the pattern seen owing to the presence of a paraprotein (monoclonal IgG component).

Table 2. Sensitivity and Specificity of Isoelectric Focusing for Multiple Sclerosis

Source	Total No. of Patients	No. of Patients With MS	Sensitivity, %
Kostulas et al ¹⁶	1114	58	100
McLean et al ¹⁷	1007	82	95
Öhman et al ¹¹	558	112	96
			Specificity, %
Beer et al ¹⁸	189	98	87
Paolino et al ¹⁹	44	26	86

Abbreviation: MS, multiple sclerosis.

serum-free light chains are readily removed by the kidney. Intrathecally synthesized IgG in patients with MS is mainly associated with κ light chains.²² Some have tried to correlate a ratio that is likely to occur in MS.²³ Free κ light chain oligoclonal bands have also been detected by IEF and immunoblotting in CSF from patients with MS but is strongly associated with the presence of IgG oligoclonal bands.²³⁻²⁵ Although this type of additional analysis may reduce the number of oligoclonal band-negative patients, the diagnostic and prognostic information conveyed by the identification of free isolated light chains, either IgA or IgM in the absence of IgG oligoclonal has not been thoroughly addressed. In fact, the isolated finding of free λ light chain oligoclonal bands is a nonspecific finding and may argue against the diagnosis of MS.^{23,25,26}

Sensitivities for detecting oligoclonal banding using IEF with immunoblotting are in excess of 95% (**Table 2**).^{11,16-19} Although we do not yet understand the precise role of oligoclonal bands in MS, their presence is tightly coupled (>95%) with the disease and, thus, can provide a useful supplement to the diagnosis. The evidence for studies from approximately 3000 patients (Table 2) is presented from individual publications using IEF with immunofixation on more than 500 patients for sensitivity and for specificity using prospective studies with follow-up exceeding 2 years. Specificities vary depending on how sure one is that there is no other reason for intrathecal inflammation. Still these are high, namely, more than 86% (Table 2). Rarely will quantitative IgG analysis findings be elevated in the absence of oligoclonal bands using IEF with immunoblotting

in a true case of MS, whereas the converse is commonly true. Zeman et al²⁷ of the Queen Square group found 34 cases that were oligoclonal band negative when the diagnosis of MS was considered likely by the clinician. After further study only 3 patients with clinically definite MS were found to be oligoclonal band negative. Thus, when the clinical suspicion is high and the test comes back negative for local synthesis of oligoclonal bands, this should be an "alert" to the clinician to reassess the case. That means that more times than not a negative test result is more likely to point to another disease than be falsely negative.²⁸ In these days where a diagnosis of MS frequently leads to initiation of cumbersome and expensive therapies, it is vital that neurologists use all the information available to assure a correct diagnosis.

There are some special considerations in CSF-serum sample pairs where the CSF but not the serum sample demonstrates a single band. In a group of such patients who underwent subsequent follow-up lumbar punctures, nearly one third converted to an oligoclonal band pattern as early as 6 months later.²⁹ These converters fell into 2 groups, either those with early disease (ie, clinically isolated syndromes) or those with progressive disease (see the following). Of the nonconverters, many were diagnosed as having alternative disorders. Therefore, although negative by definition, a single CSF band may be a good reason for repeating CSF analysis, unless other criteria clearly point to MS. This study²⁹ also verifies why oligoclonal bands have the highest specificity for MS and a single band is not diagnostic.

Cerebrospinal fluid analysis is the first criterion for making a diagnosis of primary progressive MS (PPMS). Although, by definition, all of the Queen Square group's cases had primary progressive MS and positive oligoclonal bands, other groups did not find this high prevalence when a clinical definition (eg, Schumacher criteria) was applied.³⁰ One laboratory found that only 83% of patients with clinical primary progressive MS were positive for oligoclonal banding, though this was using the less sensitive polyacrylamide gel electrophoresis-IEF system. Few other large studies exist. In the largest study to date in primary progressive MS involving more than 900 patients (the PROMISE study [Jerry Wolinsky, MD; unpublished data; 2004]) up to one third of patients were said to be CSF negative; however, CSF analysis was not controlled and many positive or negative definitions were based only on quantitative IgG analyses.

There have been several studies looking at the utility of CSF analysis in patients suspected of having MS on the basis of experiencing a "clinically isolated syndrome" and having suspected typical MS lesions on MRI imaging.³¹⁻³⁴ When using the high-caliber CSF assays such as described herein, patients who present with a clinically isolated syndrome and who have a normal MRI and normal CSF analysis findings have a low probability of developing MS.³⁵ This high negative predictive value should encourage the neurologist to consider other diagnoses to account for the clinically isolated syndrome presentation. Furthermore, in cases of a clinically isolated syndrome where the MRI is either negative or shows only nonspecific lesions, the CSF can be positive in more than 25% of individuals.^{33,35,36} This would further encourage neurologists to follow up such patients for the development of new MRI lesions or clinical symptoms and signs of MS.

NATURE OF THE CSF IMMUNE RESPONSE

It is unlikely that each IgG oligoclonal band seen represents the product of a single B-cell clone and that if gels could be further resolved (eg, 2-dimensional gels), oligoclonal bands would probably separate out into several different clones. To date, there has been no definite association of these oligoclonal bands with any consis-

tent antigen in patients with MS. It is clear^{9,12,37,38} that intrathecal antibody synthesis against many different antigens contributes to the IgG oligoclonal bands in CSF, either detected by antigen-driven immunoblots¹² or by quantitative detection with the antibody index.^{9,37} But, to date, there has been no definite association of these specific antigens with the cause of MS. Some particular observations, such as the high frequency (despite low intensity) of intrathecal antibodies against neurotropic viruses in MS^{9,37} need further discussion. It is also clear that many of these antibodies are low affinity.^{12,39} That the same pattern has been seen consistently in an individual over time suggests a long-lived chronic intrathecal immune response that is seemingly unique to each individual.^{14,32} It is also impossible to eliminate these bands following intensive immunosuppression aiming at complete immune ablation, such as that involved in studies of autologous bone marrow transplantation in MS.^{40,41}

Elevated immunoglobulin levels and oligoclonal banding indicate localized B-cell expansion in the brain. Analysis of the immunoglobulin heavy chain repertoire in CSF-derived B cells has demonstrated both clonal expansion and the process of somatic hypermutation.⁴² Recent studies using single-cell polymerase chain reaction to analyze both heavy and light chain rearrangements demonstrated not only clonal expansion of B-cell clones but also that receptor revision had probably occurred. As new techniques in molecular biology are applied to the study of CSF B cells in MS, it is likely that the phenomena of elevated immunoglobulin levels and oligoclonal bands may provide new opportunities for understanding the pathogenesis of this disease.

SUMMARY AND RECOMMENDATIONS

After reviewing all the information available on the quality, sensitivity, and specificity of CSF analysis for a diagnosis of MS, this committee drew up a series of conclusions and recommendations. The "new diagnostic criteria"¹ for MS have established CSF testing as an integral part of making a diagnosis of MS. This committee, including some of the foremost experts in CSF analysis, took as its principal mandate to decide on the most acceptable approach today toward the use of CSF as part of the workup in a patient suspected of having MS. The committee has agreed to 12 recommendations regarding the analysis of CSF so that neurologists know what to expect and laboratory medicine specialists should seek to maintain a minimal acceptable standard (**Table 3**).

1. The single most informative analysis is a qualitative assessment of CSF for IgG, best performed using IEF together with some form of immunodetection (blotting or fixation). That this technique should become the gold standard has met with recent approval from the Food and Drug Administration.

2. This qualitative analysis should be performed using unconcentrated CSF and must be compared directly with a serum sample run simultaneously in the same assay in an adjacent track.

3. Optimal runs use similar amounts of IgG from paired serum sample and CSF.

Table 3. Recommendations

Test Recommendation	Comment
IEF and immunofixation (IgG)	Now the "gold standard" with greatest sensitivity and specificity for MS diagnosis
Unconcentrated CSF	Concentrating CSF leads to artifacts
CSF and serum sample run in parallel	Tracks need same IgG amounts "by eye"
Oligoclonal bands need to be unique in CSF	Represent intrathecal synthesis
Common oligoclonal bands are irrelevant	Due to artifacts from different ampholytes
Reports standardized	5 Standardized patterns are internationally recognized
Expertise required	Ability to recognize substandard runs and assure quality
Full CSF report most helpful	Groups together <i>all</i> data from routine biochemistry (protein, glucose, and lactate levels), hematology (blood cell count and differential cell count) and specialized immunochemistry (IEF, quantitative IgG, IgG index, albumin index) laboratories into a single report
Quantitative IgG analysis	Compliments IEF but rarely picks up new cases of MS not identified by the gold standard; false positive elevations of IgG index noted in cases where BCB is "leaky"
Albumin index (quotient)	Assesses leakiness of BCB barrier
Light chain analysis	Requires specialized laboratory and adds little in routine MS diagnosis
Repeat CSF analysis if clinical suspicion is high but test result is negative	CSF studies early in the course of disease might be negative but with time are positive in most cases of MS; consider repeat analysis if a single band is seen on IEF
Quality control	Both internal and external; example of standard; available at http://www.teamspace.net/CSF

Abbreviations: BCB, blood-cerebrospinal fluid barrier; CSF, cerebrospinal fluid; IEF, isoelectric focusing; MS, multiple sclerosis.

4. Recognized positive and negative controls should be run with each set of samples and the entire gel rejected if oligoclonal bands in the positive controls are poorly developed or the negative controls are overdeveloped.

5. Cerebrospinal fluid reports of qualitative analysis should be made in terms of 1 of the 5 recognized staining patterns of oligoclonal banding.

6. Interpretation should be made by an individual experienced in the technique used.

7. Neurologists need to consider the results of *all* other tests performed as part of the CSF panel (eg, cell count; protein, glucose, and lactate levels; and others).

8. In certain cases, an evaluation using light chains for immunodetection can help to resolve equivocal oligoclonal IgG patterns.

9. Consideration should be given to repeating the lumbar puncture and CSF analysis if clinical suspicion is high but results of CSF are equivocal, negative, or show only a single band.

10. Quantitative IgG analysis is an informative complementary test but is not considered a substitute for qualitative IgG assessment, which has the highest sensitivity and specificity.

11. When performed, nonlinear formulas should be used to measure intrathecal IgG levels that consider the integrity of the BCB by also measuring the ratio of albumin in CSF to serum (also known as Q_{alb} ; a measure of BCB "leakiness").

12. Laboratories performing routine CSF analysis should be those that ensure their own internal quality control and participate in external quality assessment controls to assure maintenance of a high standard of reliability and performance, as has been recommended in some international consensus papers.^{6,21} Available at: <http://www.teamspace.net/CSF>.

Given that patients need to undergo a lumbar puncture to obtain CSF, they deserve to have it analyzed in a manner that will yield the most informative results. It is hoped that neurologists will, therefore, demand that CSF

be analyzed at least in the way that was outlined earlier herein and that laboratories performing CSF testing are aware of the standards expected. The only way to accomplish this would be for neurologists to find out which laboratory will be analyzing the CSF and inquire about the assays that are used. Given that the Food and Drug Administration has acknowledged the first criterion, it should become easier to find laboratories fulfilling the outlined criteria.

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