CONSENSUS

Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report

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Abstract

The Committee of the European Concerted Action for Multiple Sclerosis (Charcot Foundation) organised five workshops to discuss CSF analytical standards in the diagnosis of multiple sclerosis. This consensus report from 12 European countries summarises the results of those workshops. It is hoped that neurologists will confer with their colleagues in clinical chemistry to arrange the best possible local practice. The most sensitive method for the detection of oligoclonal immunoglobulin bands is isoelectric focusing. The same amounts of IgG in parallel CSF and serum samples are used and oligoclonal bands are revealed with IgG specific antibody staining. All laboratories performing isoelectric focusing should check their technique at least annually using "blind" standards for the five different CSF and serum patterns. Quantitative measurements of IgG production in the CNS are less sensitive than isoelectric focusing. The preferred method for detection of blood-CSF barrier dysfunction is the albumin quotient. The CSF albumin or total protein concentrations are less satisfactory. These results must be interpreted with reference to the age of the patient and the local method of determination. Cells should be counted. The normal value is no more than 4 cells/μl. Among evolving optional tests, measurement of the combined local synthesis of antibodies against measles, rubella, and/or varicella zoster could represent a significant advance if it offers higher specificity (not sensitivity) for identifying chronic rather than acute inflammation. Other tests that may have useful correlations with clinical indices include those for oligoclonal free light chains, IgM, IgA, or myelin basic protein concentrations.

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The clinical diagnosis of multiple sclerosis can be supported by the use of laboratory techniques for the analysis of CSF. This important fact has already been acknowledged by another group of experts.[1] We have refined their notions of "oligoclonal bands or increased CNS production of IgG" and offer considered opinions on the relative importance of different approaches to the study of CSF immunoglobulins. Specifically, we have contrasted qualitative (oligoclonal bands) with quantitative (increased CNS production of IgG) methods for their sensitivity and specificity (defined later) in the diagnosis of multiple sclerosis.

There are, unfortunately, several papers on multiple sclerosis that do not give sufficient technical details of the methods used for the determination of oligoclonal bands or increased CNS production of IgG. These techniques must clearly be calibrated by each individual laboratory. To allow proper international comparisons, authors should specify what percentage of patients with clinically definite multiple sclerosis are positive with their techniques—that is, the test sensitivity. They should also state what percentage of normal or other inflammatory diseases (acute or chronic) are positive—that is, the test specificity. These are important because the percentage will be influenced by the local prevalence of multiple sclerosis and other inflammatory diseases.

There is a voluminous literature on CSF analysis in multiple sclerosis ranging from more fundamental aspects to reported changes with treatment. We thus refer to some selected reviews.[2–9] What is of more practical relevance to the neurologist is the role of CSF analysis in helping to make the diagnosis of multiple sclerosis.

Approach to consensus on single topics

Delegates were chosen from most European countries with an inclination towards those who had provided a laboratory diagnostic service by CSF analysis. The topics discussed were initially assigned to individual delegates who each prepared a discussion paper on their respective topic of interest. It was also agreed that the relative importance of each recommendation would be assigned to one of three categories of tests: (1) an *Essential* test is required to support the laboratory basis for diagnosis of multiple sclerosis; (2) *Complementary* tests provide useful additional information to further support the diagnosis,

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Frequencies of abnormal CSF variables in clinically definite multiple sclerosis

Essential test:	
Oligoclonal IgG in CSF (isoelectric focusing)	>95%
Complementary tests:	
Abnormal blood CSF barrier function ($QAlb > 7 \times 10^{-3}$)	12%
Increased IgG quotient (IgG index, IgG (local))	70–80%
Increased cell count >4/μl	50%

and (3) *Optional* tests are used in some local centres to provide additional information. Further work on the optional tests is being undertaken, however, to try to establish their eventual role in aiding diagnosis.

In practical terms we worked through three sequential drafts with each version becoming successively shorter. The principle of consensus then adopted was that if more than two people objected, the relevant statement was dropped. It was not difficult to agree unanimously on isoelectric focusing but there were obvious objections to the use of any one reference as the "chosen" technique. After much discussion about blood-brain and CSF barriers and mathematical formulations that attempt to express quantitative measures of "CNS production of IgG", all agreed that these were not essential (as opposed to isoelectric focusing). There was, however, almost unanimous agreement on non-linear formulations, which in practice would be different in various laboratories. Most other issues were eventually agreed without undue difficulties.

Diagnosis of multiple sclerosis

The diagnosis of multiple sclerosis is ultimately a clinical decision although examination of CSF among other paraclinical tests is an important guide. Perhaps the greatest attraction of CSF examination is that it can primarily show an inflammatory origin of CNS abnormalities.

Of the various tests that can be applied to CSF, those that detect a humoral immune response within the CNS are the most important in multiple sclerosis. This is because oligoclonal banding of IgG is reported in most patients with multiple sclerosis. Isoelectric focusing is the most sensitive method for detecting local synthesis when compared with all other quantitative and qualitative methods. Local synthesis is not specific to multiple sclerosis, however, as there may be oligoclonal bands of IgG in many other inflammatory neurological diseases. It is considered an essential test, whereas three others are considered complementary (table). Some optional tests are also considered.

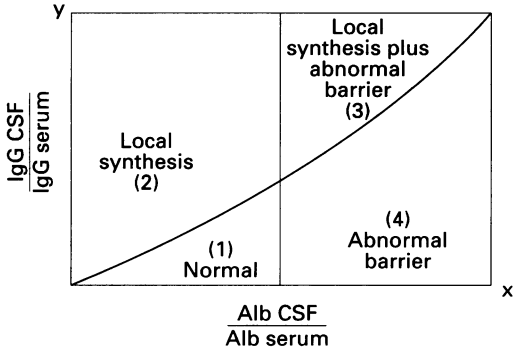

Figure 1 CSF IgG index. The y axis shows increasing values for the quotient of IgG in CSF/IgG in serum, and the x axis shows increasing values for the quotient of albumin in CSF/albumin in serum. The four areas signify: (1) normal; (2) local synthesis (normal barrier function); (3) local synthesis plus abnormal barrier function; (4) barrier function abnormal (not local synthesis).

Integrated report

When the results of several tests that can give parallel information are available, these should be presented in a clinically orientated report. The clinician may benefit if some form of quantitative graphical representation (fig 1) is used for such an integrated report. It has to be emphasised that the time of lumbar puncture with respect to the course of the disease is important for the interpretation of CSF data. In arriving at consensus for the graphical representation in fig 1, the following points were noted as showing an overall agreement: Laurell subsequently modified his original plot to show a change in slope rather than a simple straight line. Reiber and Felgenhauer devised an empirical fit to yield a hyperbolic curve, as contradistinct from Reiber's original straight line(s) model. Schuller's terms for the relevant areas are: (2) "inflammatory"; (3) "meningitic"; (4) "transudate". Sindic's term for area (4) is "mirror". Thompson's term for area (3) is "greater than".

Single topics

SAMPLE HANDLING AND CELL EXAMINATION

A defined amount (about 10 ml in adults, less in children) of CSF should be collected in polypropylene, siliconised glass, or glass tubes to allow for all tests (table). Cell numbers should be counted within 30–60 minutes of lumbar puncture. If the lumbar puncture is not performed at the same hospital as the CSF laboratory, the CSF should be transported to the CSF laboratory as soon as possible (preferably within six hours) for routine cytological examination. Differential cell loss occurs during CSF storage. The number of white cells in normal CSF is no more than 4/μl.[9]

Cytological examination is considered a complementary test in the diagnosis of multiple sclerosis. About 50% of patients with clinically definite multiple sclerosis show a normal cell count and only 1% of patients with multiple sclerosis have cell counts of more than 35/μl. Cell counts > 35/μl make multiple sclerosis unlikely, and thus other diagnoses should be considered.

Different methods such as cytocentrifuge or sedimentation chamber can be used for cytological preparation.[10] The reporting of CSF cytology to the clinician should be descriptive including, if possible, considerations of any alternative diagnosis.

EVALUATION OF THE BLOOD-CSF BARRIER

The blood-brain barrier is different from the blood-CSF barrier. By analysing the protein

content of lumbar CSF, it is possible to assess the integrity of the (functionally defined) blood-CSF barrier, but not the isolated blood-brain barrier. In healthy people or in patients without objective signs of neurological disorders, the passage of plasma proteins across the blood-CSF barrier depends on their hydrodynamic radii, which are related to their molecular weights under steady state conditions.[11] In such populations the absolute CSF concentrations of specific plasma derived proteins depend on many factors including the serum concentration of each protein, blood-CSF barrier integrity, rate of CSF flow, molecular size of the protein, age of the patient, and the volume of CSF removed.

Albumin, the major CSF protein, is synthesised only by hepatocytes and is not catabolised within the CNS. Dynamic studies with intravenously injected radiolabelled albumin[12] have shown that serum is the source of CSF albumin and strongly support the use of CSF/serum albumin quotients (QAlb = CSF albumin/serum albumin) to assess the blood-CSF barrier function. Another approach[13 14] is related to the measurement of CSF albumin only: any increase of CSF albumin (above the mean) indicates altered blood-CSF barrier function.

Determination of total CSF protein is less reliable than that of CSF albumin. If total protein is adopted as an alternative to albumin, then the method used should yield a similar optical density/g protein for both albumin and IgG. This is necessary in view of the wide variability of IgG relative to albumin, which can vary between 3% in normal and 30% in abnormal CSF. The IgG ratio (CSF IgG/CSF albumin) is of limited value as a parallel serum specimen should be analysed (see later).

The albumin quotient is age dependent.[15 16] The upper reference limit for the first 10 ml of lumbar fluid is $5{\cdot}0 \times 10^{-3}$ for patients under 15 years of age; $6{\cdot}5 \times 10^{-3}$ for patients aged 16–40 years; 8×10^{-3} for patients aged 40–60 years and $8\text{–}9 \times 10^{-3}$ for patients over 60 years. Most patients with multiple sclerosis have values for the albumin quotient below the upper reference limit. Higher values suggest a different neurological disorder.

Transudated CSF immunoglobulins, as calculated by a CSF/serum quotient, are not linearly related to the albumin quotient in cases with blood-CSF barrier dysfunction. The use of non-linear formulae or graphs for the interpretation of IgG values is therefore recommended (fig 1).

To minimise analytical imprecision, the CSF and serum concentrations for each particular protein should be analysed by the same method and within the same analytical series.

QUANTIFICATION OF THE HUMORAL IMMUNE RESPONSE IN THE BRAIN

It is a necessary requirement for any quantitative assay that each laboratory must establish its own reference range for particular protein tests.

The detection of a humoral immune response in the CNS requires an expression of results that will discriminate between blood derived and brain derived immunoglobulin fractions in CSF. Such quantitative expressions are based on calculations of the CSF/serum quotients.[12 14 17–19] These quotients are also used for comparisons of intrathecal synthesis of all immunoglobulin classes (IgG, IgA, IgM), as well as quantitative follow up of intrathecal antibody synthesis, and calculation of specific antibody index values (also called antibody specific activity).

Use of the CSF/serum quotient for IgG reduces variation due to differences in the individual concentrations of serum IgG. By referring this CSF/serum IgG quotient to the CSF/serum albumin quotient it is possible to further reduce the variation of the IgG quotient related to individual differences in blood-CSF barrier function. There are many approaches by which both of these quotients are combined to obtain an expression that will discriminate between the locally synthesised IgG fraction in the brain and the fraction of CSF IgG that is derived from the blood by filtration.

The use of a non-linear relation between the IgG quotient and the albumin quotient is recommended, as a linear approach can lead to a loss of sensitivity when there is blood-CSF barrier dysfunction.[19–22] This is especially true for larger molecules such as IgA or IgM. If quantitative values are reported, the graphical representation of the immunoglobulin quotients (fig 1) as a function of the albumin quotient is recommended as this gives simultaneous information about any local humoral immune response or any blood-CSF barrier dysfunctions. Measurements of IgM and IgA indices are optional tests currently under study to investigate whether they provide additional useful information.[14 23]

Another optional test under study is the detection of intrathecal synthesis of specific antibodies (for instance, against the measles virus). These specific antibody tests have gained further clinical relevance through improvement of the sensitivity of the evaluation techniques, mainly by the introduction of extrapolation methods for the locally synthesised fraction of IgG (or IgM), as well as correction for any blood-CSF barrier dysfunction.[14 24–26] Of special interest for the diagnosis of multiple sclerosis is the report that polyspecific antibody synthesis against several different viruses can occur in the brain. In one series, local synthesis of antibody to measles, rubella, and/or zoster could be found in the CSF of 94% of patients with multiple sclerosis.[26] The relevance of these methods has been partially confirmed by the identification of oligoclonal patterns on isoelectric focusing for each virus (measles, rubella, zoster) by use of the affinity mediated capillary blot technique.[27] Also, it has been found

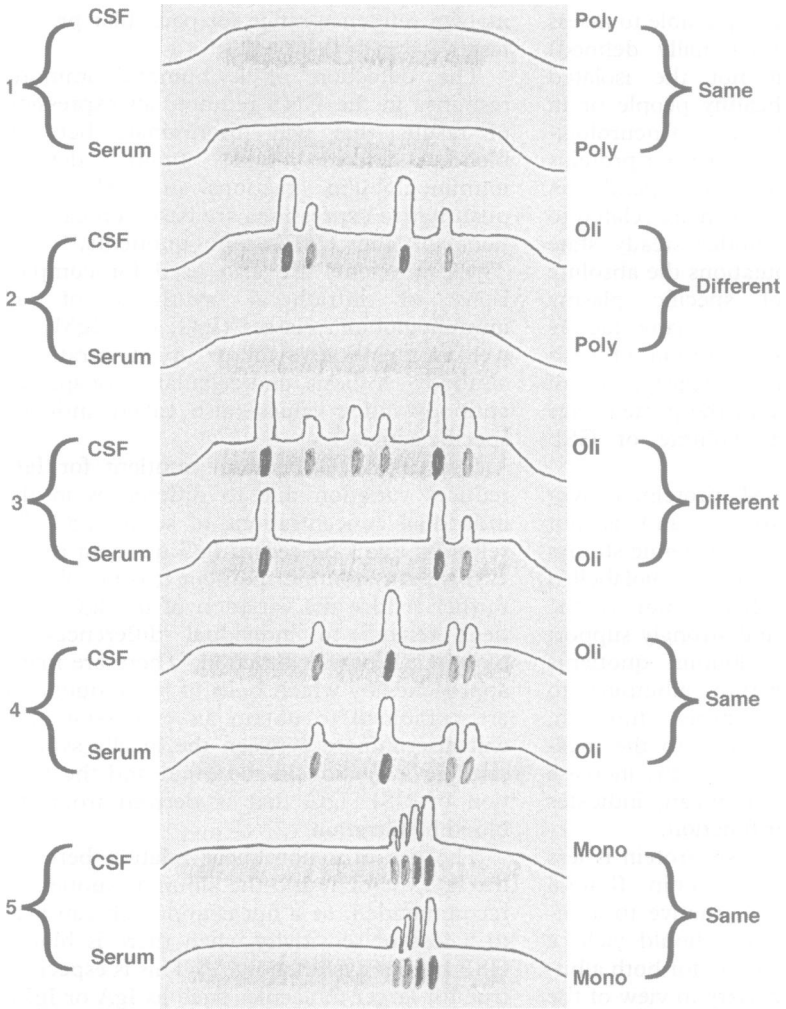

Figure 2 Diagram showing idealised CSF and serum isoelectric focusing patterns. Different CSF/serum patterns denote local IgG synthesis. Densitometric scans of the patterns show the differences in optical density (relative amounts of protein per band). Type 1 is normal. Type 2 is found in multiple sclerosis. Type 3 is found in multiple sclerosis and brain inflammation in systemic disease—for example, sarcoidosis. Type 4 is found in systemic inflammation—for example, Guillain-Barré syndrome. Type 5 is found in myeloma or monoclonal gammopathy of uncertain significance; The pH gradient is from 6–9 and the cathode is on the right; Poly = polyclonal; Oli = oligoclonal; Mono = monoclonal.

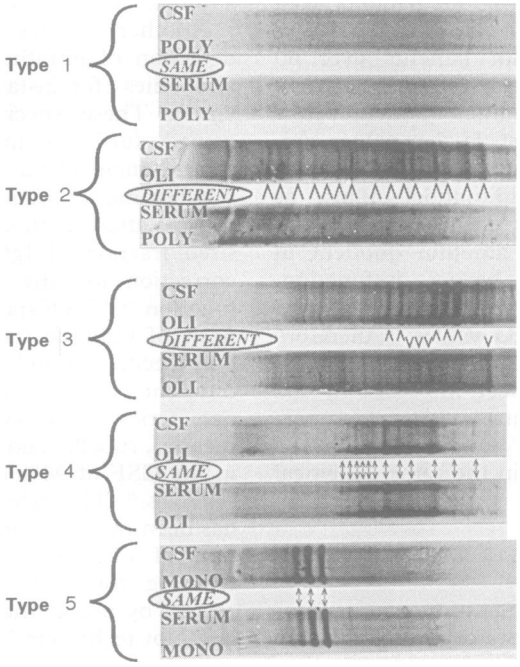

Figure 3 CSF and serum isoelectric focusing patterns. Different CSF/serum patterns denote local IgG synthesis. Typical examples of the five patterns shown in fig 2. Details are the same (see legend to fig 2).

that the antibody affinity is different in acute compared with chronic diseases, which further supports the idea that the polyspecific immune response may also become an important tool for diagnosis in multiple sclerosis.[28]

For the correct interpretation of the humoral immune response in CSF, it is important to keep in mind that the local IgG, IgA, or IgM synthesis, including any specific antibody synthesis in the brain, might have several origins. It could be due either to a persistent antibody response from an old clinically irrelevant immunological process or to an acute inflammatory process. Local IgG synthesis, detected either by increased IgG quotients, formulae, or by isoelectric focusing, can still be seen many years after adequately treated cases of neurosyphilis or neuroborreliosis, among other examples of an intrathecal immune response.

ISOELECTRIC FOCUSING OF OLIGOCLONAL IgG

The strongest consensus is that isoelectric focusing is the most sensitive test for the detection of humoral immune responses when using the same amounts of IgG in parallel CSF and serum specimens.[13 21 27 29–39] The oligoclonal bands resolved are preferably visualised by IgG specific antibody staining. Also, useful information can be obtained concerning other proteins by means of a general protein stain.

It should be emphasised that the finding of oligoclonal bands by isoelectric focusing is not specific for multiple sclerosis. It reaches its maximal value in differential diagnosis only when other known causes of CNS inflammation have been excluded.

The significance of individual bands in CSF can only be properly understood in the context of a parallel serum specimen as well as attention to the overall band pattern of all sample tracks on the isoelectric focusing plate. Isoelectric focusing can be simply thought of as separation of IgG on the basis of different charges or isoelectric points. It is important to exclude artefactual bands that are caused by non-linearity of the isoelectric focusing pH gradient. A good practical indicator for these is to compare the serum patterns from several patients. Bands that are at the same isoelectric point in all specimens of a given run are most likely to be artefacts produced by the ampholytes used in the separation. The higher the number of these artefactual bands, the more difficult it is to recognise not only legitimate abnormal serum bands, but even CSF bands, which can be obscured by interference from the common bands. The choice of commercial source of ampholytes is more important than the choice of support media (for example, agarose *v* polyacrylamide).

Reports of CSF protein analysis for clinicians must always clearly distinguish the facts from the interpretation and qualitative from quantitative results. Under "facts" it should be clear whether the band pattern in CSF is polyclonal (no bands), monoclonal (paraprotein bands), or oligoclonal (few bands).

There must be parallel investigation of serum with a clear comment on the relative band patterns in CSF and serum. Figures 2 and 3 show examples of the five types of patterns.

The banding patterns on isoelectric focusing shown in figure 2 are simplified for purposes of demonstration. Densitometric scanning is not required for interpretation.

Figure 3 gives actual banding patterns as examples of the five types. Original patterns are always more clearly visualised than any photographic reproductions.

For the five types of band patterns, only patterns 2 and 3 represent local synthesis of IgG within the CNS. Evaluations are as follows: (1) normal CSF; (2) CSF restricted oligoclonal bands: local synthesis; (3) CSF restricted oligoclonal bands with additional, identical bands in CSF and serum: local synthesis; (4) identical oligoclonal bands in CSF and serum: not local synthesis; (5) monoclonal bands in CSF and serum: not local synthesis.

IgA

IgA analysis, either by quantitative or qualitative techniques, is of little value for the laboratory supported diagnosis of multiple sclerosis.

Strong intrathecal IgA production, however, may imply a different diagnosis. Most methods for quantitative analysis of IgA production have so far failed to take into account the relative proportion of monomeric and dimeric IgA in both CSF and serum, although dimeric IgA was shown to be preferentially produced in cases of intrathecal synthesis.[40] As a consequence, amounts of local IgA synthesis could be underestimated depending on the method used. The occurrence of oligoclonal IgA bands on isoelectric focusing in multiple sclerosis or other neurological diseases is uncommon.[39 41]

IgM

Determination of CSF IgM by quantitative and qualitative methods to show intrathecal production of IgM are optional tests for the diagnosis of multiple sclerosis. The recommended method for qualitative detection of oligoclonal IgM bands is electrophoresis or isoelectric focusing of unconcentrated CSF and subsequent immunodetection.[42] Intrathecal production has been found, by quantitative and qualitative assays, in only 30% to 60% of patients with multiple sclerosis and thus seems to be of less value than detection of oligoclonal IgG bands. A degree of clinical relevance of IgM estimation has been reported due to its decrease with duration of the disease process[43] and conversely, being more common with early exacerbations of the disease.[44 45]

Further collaborative work is required to ascertain correlations between clinical variables and other CSF indices including myelin basic protein.[29 46]

FREE LIGHT CHAINS

In multiple sclerosis, oligoclonal free light chain bands are seen with about the same frequency as that for oligoclonal IgG bands, and this detection is a complementary, although optional, test to establish a laboratory supported diagnosis.[27] Electrophoresis on polyacrylamide gel[47] or agarose[48] are alternative techniques that can be used to separate free from bound light chains. After separation, free light chains are identified by immunostaining.

The quantitative determination of free light chains is critically dependent on the specificity of the antiserum used.[49] Absolute levels of free κ and λ light chains are increased in about 80% and 60% of multiple sclerosis samples respectively.[44] The influence of both the serum concentrations and of the brain-CSF barrier on the CSF concentrations are taken into account by the calculation of index values.[49]

QUALITY ASSURANCE

Most of the standards for analytes in diagnosis from CSF are defined as method related values. For internal quality assurance it is necessary to use a reference material such as diluted serum or, much better, an accepted CSF control sample. For detection of precision a local CSF pool can be used as a daily control. External quality assessment (CSF survey) by an external agency is also necessary. The international standardising organisations have cooperated to develop a harmonised proficiency testing protocol for CSF.

Analysis of CSF has the advantage that a CSF/serum quotient can be calculated for each protein. If CSF and serum protein values are measured in the same run, the quotient eliminates many of the discrepancies due to method related calibrations. The CSF/serum quotient thus approximates to a method independent value.

The problem of a complicated quality control in cytology could be solved by sending sets of cytological slide preparations to different laboratories.

Proficiency testing in CSF analysis should also consider the control of the quality of data interpretation by including the five different focusing patterns[50] that have been widely recognised in our collaborative studies of "blind" CSF samples (data not shown).

COSTS

The costs vary from around £25 to £90 per profile for the four tests listed in table 1. There seem to be three major variables that contribute to these costs: (1) technician time: beyond the basic costs for various labour intensive techniques, there may even be on call payments—for example, in the case of cell counting; (2) reagents: these are divided mainly between the more expensive commercial preparations that are available for the various tests as opposed to the much more economical "home-brewed" reagent kits; (3) interpretation: this depends largely on the analyst having the necessary degree of experience required to recognise the five basic patterns.

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