Review Article

The clinical use of cerebrospinal fluid studies in demyelinating neurological diseases

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Summary: The clinical diagnosis of definite multiple sclerosis is supported by abnormalities in the cerebrospinal fluid: variable mild pleocytosis and elevation of total protein, moderately elevated total IgG in most patients, and the almost invariable presence of discrete immunoglobulins after electrophoresis, the oligoclonal bands. The oligoclonal bands are non-specific, and are seen in most diseases of the nervous system, but their temporal uniformity in each patient with multiple sclerosis is characteristic. Prognostically, patients with a single episode of optic neuritis or paraesthesia who have oligoclonal bands are more likely to develop multiple sclerosis than if the spinal fluid were normal. In the Guillain-Barré syndrome, the spinal fluid total protein is transiently elevated, with no pleocytosis. Oligoclonal bands are usually found in the acute phase and only persist in those patients with chronic or relapsing polyneuropathy.

Introduction

Recent advances in the study of cerebrospinal fluid (CSF) in demyelinating diseases of the nervous system may now lead to some improvements in diagnosis and prognostication. The most common demyelinating disease of the human nervous system is multiple sclerosis (MS), which has a prevalence rate of over 30 per 100,000 in high frequency areas such as the United Kingdom.¹ First described separately by a Scotsman and a Frenchman^{2,3} the diagnosis is still made largely on clinical criteria, but now supplemented with information from cerebrospinal fluid (CSF) immunoglobulins.⁴ The Guillain-Barré syndrome (GBS) with an incidence rate of 1:100,000 is an acute peripheral demyelinating disorder that is diagnosed clinically,⁵ with CSF cell and protein features that may strongly support the diagnosis. These two disorders exemplify the value of CSF analysis in the diagnosis of demyelinating diseases.

In the context of MS and GBS, lumbar puncture is relatively safe: the temporary post-lumbar puncture

symptoms of headache, nausea, vomiting and low back pain occur in less than one third of all patients.^{6,7} Occasionally, the GBS is associated with intracranial hypertension and papilloedema. In this situation, the lumbar puncture is preferably performed after a computed tomographic (CT) scan (to exclude an unsuspected intracranial pathology) and in liaison with neurosurgical colleagues in case of subsequent clinical deterioration. The reader is referred to a standard text for details of the correct procedure to obtain CSF by diagnostic lumbar puncture.⁶

Normal ranges for many CSF components are described in this review, but there will be slight variation between laboratories because different methods are used. Furthermore, the patient's blood must always be examined to differentiate the CSF changes that are independent of blood (which occur in MS and GBS) from the systemic changes that occur in systemic infections and gammopathies.⁸ Abnormal CSF immunoglobulins are of great value in demyelinating disorders, but only when normal in the patient's blood. An occasional traumatic lumbar puncture will occur even with the best techniques, and this prohibits reliable diagnostic information. In these circumstances, a repeat examination may be considered after 3–

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10 days, by which time the changes secondary to the blood leakage should have resolved. For immediate patient management, however, it is useful to make approximate allowances for the blood leak prior to the second examination, and estimates of the CSF/blood contribution for cells and protein may be calculated:⁹ a useful rule of thumb is that if the patient has a normal blood profile, the blood can be expected to contribute one white blood cell (WBC) per 700 red blood cells (RBC), and 0.01 g/l of total protein per 1000 RBC; further allowance must be made if the patient has an abnormal blood picture. This derived data is most useful in the acute GBS situation, when it enables the clinician to at least make an estimate of the true CSF values for WBC and protein.

CSF abnormalities (none are diagnostic in isolation) in MS and GBS will be discussed in the context of other neurological disorders with regard to their diagnostic sensitivity and specificity, as well as to their prognostic value. This review will not discuss in detail the CSF changes in distinctively non-demyelinating disorders, such as acute infections or haemorrhage in the nervous system.

CSF in clinically definite MS

In MS, there is an increase in the WBC count in one third of patients, while the remainder are normal.⁶ Ninety five per cent of patients have less than 16 WBC/mm³,¹⁰ but occasionally values over 100/mm³ may be recorded. Two-thirds of MS patients have a normal CSF total protein concentration (0.1–0.5 g/l), and a mild elevation occurs in the other patients. An elevation of greater than 1 g/l may occasionally occur in active spinal MS, but even then it is prudent to consider an alternative diagnosis.

The total CSF IgG is usually elevated in MS, while serum immunoglobulins are normal. To determine that an increase in CSF immunoglobulin is not derived from the serum, it is compared with one of the other major proteins that is considered constitutive (i.e. produced in constant quantity, unaffected by disease). There are two formulae that are used commonly. One is the IgG/albumin index,¹¹ which is calculated: $\text{IgG (CSF)} \times \text{albumin (serum)} / \text{IgG (serum)} \times \text{albumin (CSF)}$. Abnormally elevated results from this index occur in 88% of MS patients compared to 18% of patients with other neurological diseases.¹² The other useful formula, developed by Tourtellotte,¹³ calculates the rate of synthesis of IgG within the blood-brain barrier: while 92% of patients with clinically definite MS have an abnormal IgG synthesis, so do 4% of normal persons. Whitaker¹⁴ emphasizes the difficulty that is inherent in both formulae: there is no precise measurement of blood-brain barrier disruption.

Probably the most sensitive and useful CSF test in

suspected MS is electrophoresis of proteins to identify oligoclonal bands (OB), (reviewed by Thompson).¹⁵ The principle involves separation of similar amounts of CSF and serum immunoglobulins in varied matrices (agarose, cellulose and acrylamide) to identify discrete bands of abnormal immunoglobulin in CSF that are absent in serum (Figure 1). The origin of the CSF OB is unknown. They may arise from a specific, as yet unidentified antigen, multiple antigens, or independent from any antigen. Primary amino acid sequence homology has been shown between several viruses and myelin basic proteins,^{16,17} and this provides new insight into the viral/autoimmune phenomena in MS.

With silver staining for protein detection, it is possible to identify OB with only 10 µl of unconcentrated CSF.¹⁸ It should be noted that results of OB investigations will, by necessity, vary depending on the methods used (e.g. OB on agarose gels stained non-specifically with Coomassie blue will be different from OB on nitrocellulose that are stained with specific antibodies).

Oligoclonal bands are present in over 90% of MS patients. The remaining few patients with definite MS who do not have OB have been shown to have very few or no plasma cells in the meninges or adjacent plaques at autopsy.¹⁹ Unfortunately, the same changes occur in a varying percentage of patients with all other inflammatory neurological diseases and, rarely, in patients with non-inflammatory neurological diseases, such as neoplasms, vascular disease, motor neurone disease, Alzheimer's disease, epilepsy, alcoholism and idiopathic vertigo.²⁰

CSF OB usually differ between patients with MS, but they remain remarkably constant for most patients, even over 10 years,^{21,22} even after immunotherapy.²³ The origin of this persistent and relatively unchanging OB response is unclear, but this phenomenon, in addition to the temporal separation of multifocal lesions, is useful in distinguishing chronic inflammatory diseases, such as MS and subacute sclerosing panencephalitis (SSPE), from acute post-infectious demyelinating diseases and GBS, in which the presence of OB is transient.

Interpretation of OB in definite MS is at present mainly limited to establishing the diagnosis,¹³ but because of their non-specificity, the results must be interpreted in the context of the clinical diagnosis. Furthermore, in definite MS, there is no correlation between the number of OB and the disease activity, severity or prognosis. A report of 10 patients who were incorrectly diagnosed as having MS²⁴ well illustrates an application of CSF analysis: all six mis-diagnosed patients who had CSF examined showed no abnormal immunoglobulin. Therefore, all patients with suspected MS who have normal CSF immunoglobulins should have other diagnoses rigorously excluded.

Figure 1 Proteins from serum (a and c) and CSF (b and d) were focussed at their isoelectric points in an agarose gel and stained with Coomassie blue. Gels a and b were from a patient with multiple sclerosis and gels c and d were from a normal person. The arrows indicate bands, oligoclonal bands, that are present in the CSF, but absent in the serum of the MS patient. These bands were confirmed as immunoglobulin G by transferring them to nitrocellulose, where they stained positively with anti-IgG antiserum.

CSF in suspected early MS

Of greater clinical value than aiding in the diagnosis of clinically definite MS, is the use of CSF studies to provide more diagnostic information in cases of possible early demyelination. Patients with isolated optic neuritis, who also have OB, have a significantly greater chance of developing MS than patients without OB.²⁵⁻²⁹ However, of those patients without OB, a small percentage also develop MS. Thompson *et al.*³⁰ recently reported that in suspected MS, significantly more patients who have initial OB develop further clinical episodes compared to those without OB. In the vague clinical category of patients with monosymptomatic sensory symptoms in the context of possible early MS, greater than 50% of patients have OB.³¹ Nine of these patients progressed to clinically definite MS over a mean interval of 13 months, whereas none of the patients without OB progressed that far. Thus, overall, the presence of OB in suspected early demyelination leads to a significantly greater chance of developing MS.

Furthermore, it has been shown that in higher risk situations, OB were always found in monozygotic twins discordant for MS,³² and were present in 18% of 'healthy' siblings of MS patients;³³ both examples suggest strong evidence of subclinical disease. There is an important distinction, however, between such subclinical disease and clinical MS: while extreme abnormalities may be apparent in the CSF and in brain magnetic resonance images,³⁴ it would be incorrect to diagnose MS in the absence of clinical disease. Similar caution is also required in discussion with the patient regarding the increased risk of definite MS developing when OB are present in possible early MS.

CSF in GBS

Changes in GBS are highly dependent on the timing of the lumbar puncture in relation to the disease process, in contrast to the situation in MS where the CSF changes are more constant over time. When the patient presents with GBS, typically there are no excess white blood cells, in conjunction with an elevated CSF total protein, but pleocytosis occurs in 20% of cases.³⁵ Values greater than 50 monocytes/lymphocytes per ml, or the presence of polymorphonuclear lymphocytes, cast doubt on the diagnosis. An elevation of total CSF protein is characteristic, though not invariable, in GBS, and is often between 1-5 g/l. This rise occurs after the first week of symptoms, and may be detected with serial lumbar punctures. Raised immunoglobulins, IgG index and the appearance of oligoclonal bands occur in the majority of patients. These, however, are usually transient and only remain

abnormal in the few patients that develop chronic disease.³⁶

CSF in other demyelinating diseases

Demyelinating diseases, in general, all show the same CSF changes that occur in MS. The only distinguishing features are the different clinical presentations and the temporary nature of the CSF abnormalities in the acute demyelinating conditions (such as post-infectious syndromes, acute disseminated encephalomyelitis). In the chronic conditions, such as subacute sclerosing panencephalitis, there is persistent CSF abnormality that is not distinctive clearly from that in MS, except for the characteristic elevated measles titres in sub-acute sclerosing panencephalitis.

Interpretation of certain non-routine CSF tests

Acute and convalescent viral serology is appropriate in the context of infections associated with demyelinating diseases, when it may establish the specific infecting agent, although it is usually not of help in differentiating acute encephalitis from perivenular demyelination.³⁷ Guillain-Barré syndrome may follow a known viral, mycoplasmal infection or immunizations, but this occurs in the minority of cases and there is no evidence that such an event influences the course of the illness. Serology in MS has shown a range of elevated titres in some patients to measles, rubella, mumps, vaccinia³⁸ and, recently, lymphotropic retroviruses.³⁹

Other CSF measurements that have been useful in research, but not in routine clinical practice, include typing of subsets of lymphocytes, detection of myelin basic proteins⁴⁰ and anti-myelin basic protein antibodies,⁴¹ specific assays for immunoglobulins M, A and G, kappa/lambda light chain ratios and measurement of free light chains.^{42,43} For a given patient over time, both myelin basic protein levels and kappa/lambda ratios correlate reasonably with damage to the nervous system in MS, as determined

clinically and with magnetic resonance imaging,^{44,45} but this applies to serial measurements in a patient, which are usually only indicated in a research protocol.

The future

The following specific investigations may prove to have clinical value:

To improve quantitation, an ideal CSF protein(s) that is not present in blood, nor altered in neurological disease, should be identified, which acts as an internal standard between patients. To correct for blood-CSF contamination, an ideal serum protein(s) should be used that is normally absent in CSF, but that is detectable in CSF in a quantity proportional to serum contamination. With over 300 proteins now identified in CSF⁴⁶ and more in serum,⁴⁷ such progress appears feasible.

In MS, there is considerable clonal uniformity and restriction of the abnormal immunoglobulins.^{21,48} Further molecular characterization of these immunoglobulin clones may lead to greater distinction between MS and other diseases.

Identification of non-immunoglobulin protein changes may help in distinguishing demyelinating diseases. A diagnostic test has been established among dementias for Creutzfeldt-Jakob disease,⁴⁹ and using the same approach in MS, 25 non-immunoglobulin alterations have been described,⁵⁰ and Walsh *et al.*⁵¹ have co-purified a protein with IgM heavy chains in MS that is absent in normal CSF.

Further comparison of OB and immunoglobulin light chains in MS with other diseases in serial samples may extend the specificity of the temporal invariance of OB noted in MS.

Having established clear evidence of subclinical disease, it will be important to investigate whether the CSF immunoglobulin abnormalities precede the pathological lesions (as identified by magnetic resonance imaging). If this were so, attempts to block such antibodies might be more strongly justified as a therapeutic procedure.

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