

The Detection of CSF Oligoclonal IgG by Isoelectric Focusing

When the clinical presentation is not diagnostic, paraclinical information is needed to diagnose multiple sclerosis.¹ The principal paraclinical investigations used are magnetic resonance imaging and cerebrospinal fluid (CSF) analysis for oligoclonal IgG. Here I describe the principles underlying the detection of oligoclonal IgG in CSF, together with examples of patterns which may be obtained and their clinical significance.

To detect oligoclonal IgG, it is first necessary to separate the individual bands from one other. Although polyacrylamide electrophoresis was used originally² to separate IgG bands, isoelectric focusing is now the standard method.³ Once separated, a sensitive method is needed to detect the bands, as they are present at low concentration in CSF. Separated bands can be detected by silver staining, but detection by Western blotting is more sensitive and specific. Rigorous attention to analytical technique is required to produce reliable results⁴, and standardised criteria are available for the performance and interpretation of CSF analysis.³

Isoelectric focusing

If a serum protein in solution is placed in an electric field it will be attracted to and migrate towards the anode (positive pole), the greater the charge on the protein, the faster it will migrate. The overall charge on a protein is determined by its amino acid composition (eg negatively charged glutamate or positively charged asparagine) and the number and structure of any negatively charged carbohydrate side chains attached to it. In practice most serum proteins are negatively charged.

In vitro it is possible to alter the charge on a protein by altering the pH of the solution in which the protein is dissolved. At low pH (high hydrogen ion concentration) carboxy groups are more likely to be protonated and therefore lose their negative charge and amino groups are more likely to gain a proton and become positively charged. If the pH of the solution were gradually lowered, there would come a point when the overall charge on the protein was zero (Figure 1). The pH at this point is called the pI or isoelectric point of the protein. If a protein was placed in a solution which had a pH equal to the pI of the protein, the protein would be uncharged and therefore would not move in an electric field. This is the basis of isoelectric focusing.

In this technique a stable pH gradient is created in a gel by the application of an electric potential to a gel containing a mixture of low molecular weight 'ampholytes'. These ampholytes contain positive and negative charges and migrate at speeds determined by their charge and therefore (as the ampholytes with the lowest pI will be the most negatively charged) they line up along the gel in the order of their pIs. The ampholytes are chosen to have pIs which span the pIs of the proteins of interest. When a protein is added to this gel it will migrate at a rate determined by its charge. At the high pH end of the gel, most proteins are negatively charged and they will therefore migrate towards the anode. The anodic side of the gel is also the side of low pH so that when the proteins migrate they enter a region of the gel of lower pH and become less negatively charged. When they reach the part of the gel which has a pH equal to their pI they stop migrating. A protein therefore migrates to the region of the gel as which it is uncharged, ie to its pI. Even if the protein starts to diffuse away from this part of the gel it will encounter a pH at which it is charged and will migrate back. Therefore very tight bands of protein are formed.

Isoelectric focusing has a high resolving power and separates proteins which may only have a very small charge difference. For instance, the carbohydrate side chains attached to a particular protein are not always the same. There may

be differences in their number and structure. These differences lead to so called micro-heterogeneity. These differences may not be picked up by simple electrophoretic techniques but can be picked up by isoelectric focusing. Thus a protein which runs as a single band on normal electrophoresis may run as several bands on isoelectric focusing.

IgG on simple electrophoresis runs as a polyclonal band. This is the result of there being many different individual molecules each at low concentration and each with a unique amino acids composition. The many overlapping bands are not resolved from one another. However, when a small number of B cell clones predominantly react in an inflammatory response, a number of individual IgG molecules derived from these clones will be present at relatively high concentrations. These IgG molecules may not be sufficiently different to be resolved by normal electrophoresis, but isoelectric focusing can resolve these bands. Indeed this was one of the earliest applications of the technique.⁵

Western blotting

Immunoglobulin in CSF is present at low concentrations and a sensitive method is required to detect it. This technique involves the transfer of all the proteins in a gel to a nitrocellulose membrane. The individual protein of interest (IgG) is detected using a specific antibody to which an enzyme (peroxidase) is attached. The location of the protein is visualised by incubation with a substrate of peroxidase which generates a coloured insoluble product.

CSF oligoclonal IgG patterns

Freedman et al⁶ identifies five classical patterns. However, there is now evidence that the classical positive pattern can be subdivided on the basis of the number of oligoclonal bands.^{6,7} The various patterns which may be observed are shown in Figure 2. Most samples show some faint background banding seen in both the CSF and serum. In practice this faint banding does not lead to any difficulties in interpretation. Different ampholyte preparations from different manufacturers behave differently in this regard.

Negative pattern (Figure 2a) - No oligoclonal bands are detected. This is the normal pattern.

Negative pattern (Figure 2b) - Similar oligoclonal bands in both serum and CSF. This pattern is found in systemic inflammatory conditions. Since CSF is essentially an ultrafiltrate of plasma, any oligoclonal IgG present in plasma will find its way into CSF. As mentioned above this oligoclonal IgG may not be apparent on ordinary serum protein electrophoresis.

Negative pattern (Figure 2c) - Similar oligoclonal bands in both serum and CSF. This pattern indicates the presence of a serum monoclonal band such as is found in association



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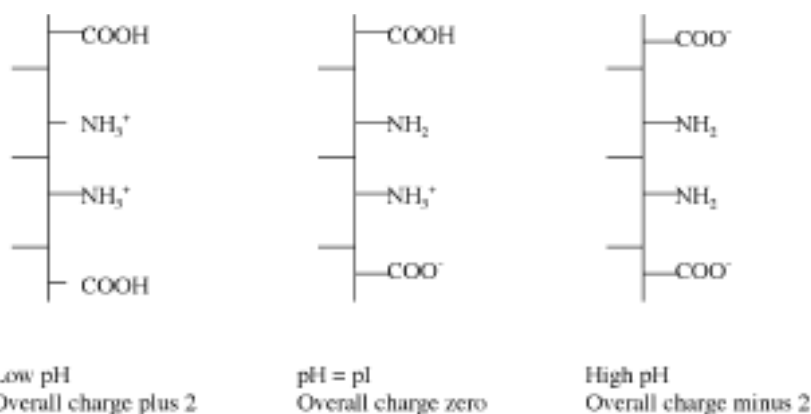


Figure 1: Effect of pH on the overall charge of a protein.

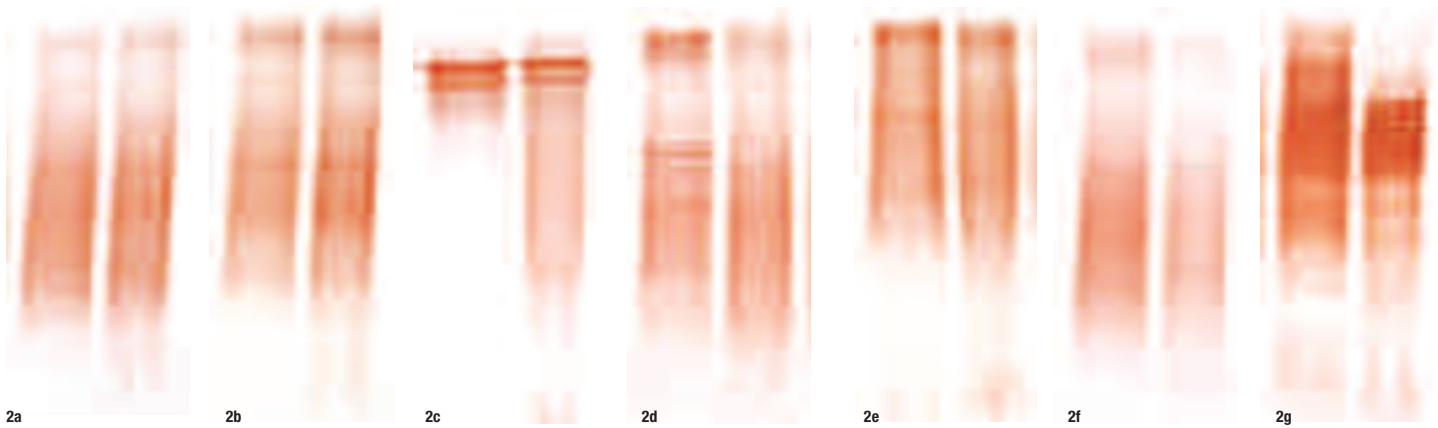


Figure 2: Examples of patterns of csf oligoclonal banding. In each pair the left hand lane is csf and the right hand lane is serum diluted 1 in 300.

with myeloma. The greater resolving power of isoelectric focusing resolves the single band found in serum protein electrophoresis into several bands which differ as a result of different degrees of glycosylation.

Positive pattern (Figure 2d) - There are multiple IgG bands. This pattern, in which there are more than ten bands unique to the CSF is highly specific for MS. Only 7 of 593 patients with neurological disease other than MS had this pattern⁶ giving a specificity of 99%. However only 46% of patients with MS have this pattern.

Positive pattern (Figure 2e) - There are fewer than ten but more than three unique bands. This pattern has a sensitivity of 85% and a specificity of 92% in MS.⁶

Positive pattern (Figure 2f) - A single unique

band in CSF. About a third of patients with this pattern go on to develop typical oligoclonal bands. About a quarter revert to normal on follow up. The rest are associated with a variety of non-demyelinating conditions which may include cerebral lymphoma.⁷

Positive pattern (Figure 2g) - Oligoclonal bands in both serum and CSF with some unique csf bands. This again supports the diagnosis of MS. This figure illustrates a sample from a patient with MS in whom there was a monoclonal band in the serum as well as unique bands in CSF.

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