A comparison of cellulose acetate immunofixation with polyacrylamide gel electrophoresis for the detection of oligoclonal bands in unconcentrated cerebrospinal fluid

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SUMMARY Two methods of electrophoresis for the detection of oligoclonal bands in unconcentrated CSF were compared. A sample of 98 routine CSFs yielded 18 positives by polyacrylamide gel electrophoresis (PAGE) while cellulose acetate electrophoresis with immunofixation (CAIF) gave 13 positives (72% of the PAGE findings). Despite the loss of sensitivity the cellulose acetate electrophoresis was easier to interpret and more suited to a routine hospital laboratory.

Electrophoresis of cerebrospinal fluid (CSF) to detect oligoclonal immunoglobulins is a useful test in the investigation of neurological disease. Polyacrylamide,¹ agar^{2,3} and agarose^{3–6} gels and cellulose acetate^{7,8} have all been used as support media with varying success. Cellulose acetate is reported to have poorer resolution than the gels.⁸ Many investigators have concentrated their CSF samples before electrophoresis although it has been shown this may alter the electrophoretic pattern.⁷ Thompson¹ therefore applied relatively large volumes of CSF directly to PAG disc electrophoresis. This technique gives good results in experienced hands but those unfamiliar with PAGE may have difficulty with the interpretation.

Electrophoresis of unconcentrated CSF on cellulose acetate is feasible when Nigrosine stain is used.^{7,9} However γ globulins stain weakly and β and γ trace globulins tend to confuse the picture when looking for oligoclonal bands.^{3,6,8} Immunofixation with antihuman IgG antisera further intensifies the γ globulin staining and removes interference from other proteins.^{6,10}

The detection of oligoclonal bands in unconcentrated CSF on cellulose acetate with immunofixation was compared with PAGE.

Material and methods

SAMPLES

Ninety-eight unselected routine CSF samples on

Accepted for publication 23 May 1983

which PAGE had been performed at the Institute of Neurology were sent to Southampton for electrophoresis on cellulose acetate. A further selected group of 13 positive and two negative CSF samples was later sent. All samples were analysed and evaluated blind.

POLYACRYLAMIDE GEL ELECTROPHORESIS

PAGE was performed by the method of Davis.¹¹ Using 7% polyacrylamide but without a stacking gel, Thompson,¹ two gels were run for each CSF. One was loaded with 100 μ g total protein and the other with 200 μ g. The first gel was stained with Coomassie blue and the second with naphthalene black. Immunoglobulin bands were identified by their greater relative affinity for Coomassie blue than naphthalene black.

CELLULOSE ACETATE ELECTROPHORESIS

A method for detecting Bence Jones protein in unconcentrated urine¹⁰ was modified by using anti γ chain antiserum in place of anti-light chain antiserum.

Cellulose acetate electrophoresis was performed on Titan III mylar backed cellulose acetate plates with a Tris-barbital buffer $i = 0.05$, pH = 8.6 using standard applicators and other electrophoresis equipment all from Helena, Beaumont Texas, USA.

Prior to soaking in electrophoresis buffer, a line is drawn on the mylar backing of immunofixation plates in waterproof ink 32 mm from the cathodic end. This can be seen through wet cellulose acetate

Fig. 1 Cellulose acetate electrophoresis stained for total protein. Samples from left to right 1, 3-5 positive, 2, 6 negative.

Fig. 2 Cellulose acetate electrophoresis after immunofixation. Samples from left to right 1, 3-5 positive, 2, 6 negative.

and guides the positioning of the antiserum strip. CSF ($5\mu\text{l}$) is placed in each sample applicator well, but by a double application with the Super Z applicator only $0.3\mu\text{l}$ of CSF is loaded onto the plate. Duplicate sets of plates were run. After electrophoresis at 180 V for 15 min the plates to be stained for total protein were fixed in TCA 100 g/l for 30 min before staining in Nigrosine 50 mg/l in

acetic acid 50 ml/l for 30 min . Following a 30 min wash in dilute acetic acid 50 ml/l , the plates were blotted and air dried.

The plates for immunofixation were placed in a humidified chamber after electrophoresis. Antihuman γ chain antiserum Dako (Mercia-Brocades Ltd, West Byfleet, Surrey) was diluted $1/3$ in physiological saline. Dilute antiserum ($250\mu\text{l}$) completely

moistens a 25 × 55 mm strip of cellulose acetate (Cellogram) which was then placed over the γ region of the electrophoresis plates—that is, anodically of the line previously drawn on the back. After 15 min the antiserum strips were discarded and the electrophoresis plates washed for one hour in detergent buffer, NaCl 9.0 g/l, Na barbitone 5.0 g/l, Triton X 100 1.0 g/l, to remove unfixed proteins. The plates were then stained with Nigrosine as described for the total protein plates. The cellulose acetate plates were viewed dry, uncleared by transillumination.

Results

Unconcentrated CSF when stained for total protein after electrophoresis gives a clearly discernible pattern from the albumin to β globulin region but γ region is diffuse and relatively poorly stained. Only the more intense oligoclonal bands can be unequivocally identified. (Fig 1) Immunofixation intensifies and clarifies staining in the globulin region (Fig 2) so that a single band can be taken as evidence of oligoclonal antibodies. Of 98 unselected routine CSF samples investigated 18 had oligoclonal bands by PAGE while only 13 (72% of PAGE findings) showed banding on CAIF. Of an additional selected group of 13 positive and two negative CSFs by PAGE, nine (69% of PAGE findings) positives were obtained by CAIF. At no time did CAIF give a positive result with a sample that was negative by PAGE.

Discussion

These results are consistent with the greater resolving power of PAGE over cellulose acetate in the detection of oligoclonal bands in CSF. However many laboratories do not have the time, expertise or equipment to run PAGE, while most laboratories already run some cellulose acetate electrophoresis and hence have the potential to run CAIF. Another alternative would be agarose gel electrophoresis followed by immunofixation which takes slightly longer and is slightly more involved than CAIF.⁴ There is moreover a considerable saving of time with CAIF, the whole procedure taking only 3½ h. There is no need to concentrate samples and as little as 5 μ l CSF may be used. The interpretation of CAIF is easier than with PAGE. Although the detection rate for CAIF was lower than for PAGE there were no false positives.

The CSF: serum IgG: albumin ratio has also been used for detection of MS. We cannot comment on its effectiveness relative to CAIF as no comparison has been made in this series. It has been shown that the

CSF: serum IgG: albumin ratio is inferior to PAGE in the detection of MS producing both false positives and negatives.¹² The ratio requires four immunological assays on each patient involving considerable analytical time, reagent costs and/or capital expenditure. Moreover the experience of the Institute of Neurology is that despite every effort only 20% of CSF samples arrive accompanied by a blood sample, thus only the CSF IgG: albumin or CSF IgG: total protein can be measured.

We therefore consider that CAIF would be a simple and useful additional technique for busy routine and smaller laboratories.

We thank Dr JE Middleton, Consultant Chemical Pathologist, Southampton for his advice and encouragement.

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