Recent Techniques in Cerebrospinal Fluid Examination

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The existence of the cerebrospinal fluid (CSF) has been known since ancient times, but it was Cottugno who first described it in some detail in 1764. Quoting from his treatise, "In these experiments which I made on the bodies of nearly twenty adults, and which I repeated at different times, I could draw off freely from the hollow of the spine, four, or even sometimes five ounces of water: I commonly found it very clear in such subjects, though it sometimes inclined a little to a yellow color: but in fetus' strangled in difficult labor, little as it was, I observed it to be always red and opaque." He also felt that the CSF was secreted by the arterial system, circulated in the subarachnoid space and was reabsorbed by the venous system. Matters remained in that state until 1891 when Quincke described a refinement in lumbar puncture using a needle with a pointed stiletto in it. The first extensive treatise on CSF composition was written by Mestrezat in 1912. Since then there has been very slow progress in the evaluation and interpretation of CSF even though there have been tremendous strides in the evaluation of other body fluids, particularly blood.

One of the reasons for this is a fear of lumbar puncture, a fear which is usually unwarranted. Unfortunately, this attitude permeates the medical profession almost as much as it does the lay public.

Tests such as cell counts, glucose, protein, and semiquantitative tests for globulins such as the colloidal gold test were products of the first part of this century. As new developments occurred in blood analysis these were applied to the CSF. These studies did not lead to any advances in the clinical chemistry of CSF, though they certainly had their place in furthering our understanding of brain chemistry and function.

The biggest advance in clinical CSF chemistry in the past decade has been the emergence of an abbreviated CSF immunology. This followed the development and refinement of agar electrophoresis and immunoprecipitation techniques.

The scope of this paper will include discussion of these advances and some promising studies of CSF enzymes in differential diagnosis as well as comment on a recurrent problem, that of the interpretation of bloody or xanthochromic CSF.

Blood in the CSF. Probably one of the most frequently encountered problems is the bloody CSF. Is it a subarachnoid hemorrhage or is it a traumatic tap?

One of the best tests and the one most commonly used is to do a cell count in the first tube and another in a subsequent tube, usually the third. This should be done even if the fluid looks grossly clear since about 500 cells must be present before the fluid becomes hazy. A significant change in the red blood cell (RBC) count of the fluid indicates a traumatic tap since the blood from a subarachnoid hemorrhage should be homogeneous by the time it reaches the lumbar sac. I say a "change" in the RBC, not just a decrease, since a marked increase would also indicate a traumatic tap. One should also closely observe the fluid as it comes out of the needle. Gross blood can sometimes be seen streaming in the fluid. One may
also see nonhomogeneous mixing in the fluid as it drops into the tube. In addition, a very bloody traumatic tap (greater than 200,000 RBC/mm³) will clot on standing; this will not occur with subarachnoid bleeding. Microscopic examination of the red cells is of little value since crenation may occur with both subarachnoid bleeding and a traumatic tap.

The next step if the fluid is bloody or colored should be to centrifuge the fluid and determine the color of the supernatant. One often hears the remark that the fluid was xanthochromic, yet the fluid was not centrifuged. Xanthochromia should refer to a yellow color of the supernatant and should never be used in reference to unspun CSF. This coloration is usually due to either oxyhemoglobin, methemoglobin, or bilirubin. Very rarely it may be due to other pigments such as carotene.⁶

The sequence of events appears to be: hemoglobin is released from the red cells in the subarachnoid space. This appears as oxyhemoglobin which in low concentrations has an orange-red appearance. Depending on the amount of bleeding, one begins to see a change in the coloration of the fluid to yellow within a few hours to a day. This is due to the heme being metabolized to bilirubin. In the case of a subdural or an intercerebral hematoma the fluid may take on a brownish-yellow appearance due to the presence of methemoglobin.

The supernatant fluid in a traumatic tap can give one of two results. It is usually clear and colorless since the red cells have not had time to break down. If the RBC count is high, however, (>12,000/mm³), some of the hemoglobin leaking out may be visible immediately.⁵

True subarachnoid bleeding will give different results depending on the time elapsed following the bleed. One should keep in mind that it may take a half hour for blood to reach the lumbar sac from the cortex. The CSF may be indistinguishable from a traumatic tap very shortly after the hemorrhage. In his classic text on subarachnoid hemorrhage, Walton observed xanthochromia in 7% of patients within 2 to 4 hours, but by 4 to 6 hours, 64% had it, and by 12 to 24 hours, 100% had it.⁷

There have been periodic attempts at using spectrophotometry as an aid in evaluating CSF pigments. The three major pigments have rather characteristic absorption patterns. Kronholm and Lintrup did a study on 1,250 CSF samples and were able to develop a set of formulae for determining the concentrations of hemoglobin, methemoglobin, and bilirubin. Their results showed that they could distinguish between intercerebral hematomas, subdural hematomas, and subarachnoid hemorrhage (Table 1; Fig 1).⁸

More recently, Kjellin and his group have expanded on this theme of CSF spectroscopy.⁹,¹⁰ Besides the disorders noted above, they have been able to correlate various CSF patterns with hemorrhagic infarctions, nonhemorrhagic infarctions, and emboli, as well as hematomas and subarachnoid hemorrhage. In the past they have had to rely on clinical and occasional autopsy conformation. When this was available there was good correlation (Table 2).

I do not advocate that these procedures be used in place of others for evaluation of bloody CSF, but as adjuncts which may be invaluable in deciding if a lumbar puncture was traumatic or not.

**CSF Proteins and Immunoglobulins.** There is considerable evidence that under normal circumstances the proteins of the CSF are derived from the serum.¹¹ The most notable exception is the so-called (tau) protein seen in electrophoresis which is probably serum transferrin which has lost two sialic acid residues (Fig 2). Normally, the CSF proteins are made up of albumin, β globulin and γ globulin in the fractions 0.58, 0.20, and 0.10 respectively. These get into the CSF by secretion from brain tissue as well as from the choroid plexus. Efflux of protein is presumably through bulk flow through the arachnoid granules. Increases in protein, especially albumin, can come from: 1) hemorrhage into the CSF, 2) breakdown in the blood-brain barrier, 3) elevated serum protein, 4) lesions of the choroid plexus or 5) blockage of CSF flow, for example, spinal block, and 6) an efflux of soluble proteins into the CSF with damage to brain tissue.

**CSF γ globulin or IgG also comes from the serum in normal persons.** In conditions where there is

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<tr>
<th>Table 1</th>
<th>CSF Pigments in Xanthochromic CSF</th>
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<tr>
<td>Intercerebral hematoma</td>
<td>Bilirubin &gt; Hemoglobin</td>
</tr>
<tr>
<td></td>
<td>Methemoglobin &gt; 25% total Hb</td>
</tr>
<tr>
<td>Subdural</td>
<td>Same as above, but metheme may be very high</td>
</tr>
<tr>
<td>Subarachnoid</td>
<td>Hemoglobin &gt; Bilirubin</td>
</tr>
<tr>
<td></td>
<td>Methemoglobin &lt; 25%</td>
</tr>
<tr>
<td>Traumatic</td>
<td>Same as subarachnoid though</td>
</tr>
<tr>
<td></td>
<td>Bilirubin should be negligible</td>
</tr>
</tbody>
</table>
Fig 1—Absorption pattern of CSF. A is from a patient who did not have active CNS disease. B is from a patient who presented with a history of confusion and a bloody CSF. The absence of a hemoglobin peak indicates blood was from a traumatic tap, but bilirubin indicates an older subarachnoid hemorrhage. C is from a patient three weeks after an intercerebral hemorrhage. The hemoglobin peak is over 50% metheme. D is hemolyzed blood.
an increased serum γ globulin or IgG, this will be reflected in the globulin composition of the CSF. In addition, if there is a breakdown in the blood-brain barrier, one may see an increase in the γ or IgG fraction simply because it makes up a higher proportion of the total protein in serum than it does in CSF.

There are times, though, when it appears that the increased immune globulins are coming from the brain itself. This was shown some years ago by Frick and Scheid-Seydel who used tagged IgG. In multiple sclerosis (MS) patients they showed that a large portion of the IgG was coming from somewhere other than serum. At present it is thought that it is coming from immune competent cells situated in the periphery of MS plaques. Other conditions which are felt to reflect central nervous system (CNS) IgG production with a raised CSF IgG are neurosyphilis and subacute sclerosing panencephalitis. Disorders such as meningitis or encephalitis may show increased IgG either from a breakdown in the blood-brain barrier or CNS production.

In 1964, Lowenthal applied agar electrophoresis to CSF and was able to confirm this increase in immune globulins (See Fig 2; Fig 3). Interestingly, conditions which have CNS immune globulin production usually show discrete banding of the γ fraction while those conditions in which the immune globulins originate in serum show a diffuse γ band: The next advance after agar electrophoresis was the development of immunoprecipitation methods, either electroimmunodiffusion or radial immunodiffusion where antibody to the immune globulins is added to the agar. The distance of the resultant precipitate from the origin is proportional to the immune globulin concentration. The advantage over electrophoresis is that only a few microliters of CSF are needed, and the subfractions of the immunoglobulins can be measured. The disadvantage is that only one protein fraction can be measured at a time.

Interestingly, the results by the different methods are remarkably similar. In MS some 75% to 85% of the patients show an abnormal γ or IgG level at some time in their disease. There is no relationship, however, to the stage of the disease or to the prognosis. Its value is therefore purely diagnostic. Inflammatory diseases such as meningitis and encephalitis show an increased level in some 35% to 40%, the lowest level being in viral meningitis, Guillain-Barré syndrome shows an increase in 30% to

<table>
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<tr>
<th>Verified Disease</th>
<th>Infarction</th>
<th>Hemorrhagic infarction or Hematoma</th>
<th>Mixed</th>
<th>Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarction</td>
<td>71</td>
<td>24</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cerebral Hemorrhage</td>
<td>9</td>
<td>46</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td>Aneurysm</td>
<td>—</td>
<td>8</td>
<td>8</td>
<td>82</td>
</tr>
<tr>
<td>Trauma (not subdural)</td>
<td>—</td>
<td>—</td>
<td>18</td>
<td>82</td>
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* A small number could not be interpreted, so figures do not add up to 100%.

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![Agar gel electrophoresis of concentrated CSF and uncentrated serum.](image)

**Fig 2**—Agar gel electrophoresis of concentrated CSF and uncentrated serum. Note presence of a prealbumin and a tau band in CSF which is not present in serum. Also note very broad gamma band.
Fig 3—Agar gel electrophoresis of CSF from two patients with elevated gamma fraction. The first is a patient with acute lead encephalopathy. The diffuse gamma band is indicative of a serum source while the multiple bands from the multiple sclerosis patient indicate a CNS source.

50%. Agar electrophoresis shows that this is a serum pattern. In other diseases such as degenerative disease, tumors, and vascular disease, the percentage with increased γ or IgG levels is quite low, though in these conditions the literature does vary a great deal and probably reflects variations in the integrity of the blood-brain barrier (Table 3).

CSF Enzymes. The measurement of CSF enzyme levels has not yielded the kind of information which was hoped for. The ones measured most commonly, lactic dehydrogenase (LDH) and glutamic-oxalacetic transaminase (GOT), appear to be elevated whenever there is significant disruption of brain tissues and do not seem to be specific for a given disease. The LDH is also increased when there is an increase in CSF polymorphonuclear cells. This relation to tissue destruction is shown by the results from a patient with recurrent strokes. Using LDH isoenzymes, it is of interest to note the transient shift to the more anaerobic forms of LDH during the acute phase (Fig 4).

CSF LDH has been noted to be elevated in meningitis of bacterial origin, but rarely in viral. This elevation has been used to try and differentiate partially treated bacterial from viral meningitis. For technical reasons the results were not really conclusive, but it did indicate a differentiation could be made by an elevated level in bacterial disease.

More impressive was a study on CSF lactate before, during, and after treatment. Bacterial disease showed an elevated lactate level until after full treatment while aseptic meningitis was the same as the controls. CSF pH showed the inverse pattern as would be expected.

While further studies will be necessary to confirm these results, the outlook for a test to distinguish partially treated bacterial disease from a viral meningitis looks promising.

Table 3

<table>
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<tr>
<th>Conditions Giving Elevated CSF Gamma Globulin</th>
<th>Conditions with Increase in CSF Immune Globulins</th>
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<tbody>
<tr>
<td>Systemic Conditions with Increased CSF Immune Globulins</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>Multiple Sclerosis</td>
</tr>
<tr>
<td>Myeloma</td>
<td>CNS Lues</td>
</tr>
<tr>
<td>Diseases with breakdown BBB</td>
<td>SSPE</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>Vasculitis*</td>
</tr>
<tr>
<td>Infection</td>
<td>Infection*</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>Sarcoidosis*</td>
</tr>
</tbody>
</table>

* In these three it is uncertain if the origin of the gamma globulin is serum or CNS or both.

Table 2 is adapted from Kjellin and Söderström, *J Neurol Sci* (23:359–369, 1974).

REFERENCES


