A membrane filter technique for cerebrospinal fluid cytology

Technical note

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A simple membrane filter technique is described for the collection and demonstration of exfoliated neoplastic cells in the cerebrospinal fluid (CSF). Abnormal cells were demonstrated in 30 cases: in four of these the diagnosis of a benign intracranial tumor was possible. Using this technique, it appears that cells from the majority of neoplasms in contact with the CSF will be demonstrable. This new technique is superior in many ways to the conventional concentration methods of centrifugation, flocculation, sedimentation, or filtration now used. The primary advantages are the simplicity, the bedside availability, the ability to reuse the filtered fluid for additional studies, the preservation of the cells for later analysis should a cytological laboratory not be immediately available, and, of primary importance, the excellent preservation of cellular morphology.

KEY WORDS : brain tumors · cerebrospinal fluid cytology · membrane filter

Papanicolaou's9 contribution to the field of exfoliative cytology in the 1940's proved of great value in the diagnosis of neoplasms of the respiratory, gastrointestinal, urinary, and reproductive system. The technique, however, was not commonly applied to the nervous system; in fact, since 1903 when the cytological examination of cerebrospinal fluid (CSF) was reported by Ravaut, et al.,10 fewer than 200 cases of abnormal cell recovery from the cerebrospinal fluid (CSF) had been published (by 1969).11 Extensive use of this diagnostic tool in the evaluation of patients with neurological disease has been hindered by the difficulty encountered in concentrating the scant number of abnormal cells that are shed into the CSF, while at the same time preserving their morphology.

Concentration of cells in the CSF may be achieved by various methods involving centrifugation, sedimentation, flocculation, or filtration. At present, centrifuged air-dried smears are most often used in cytological examination. Although giving rapid and effective sedimentation, centrifugation per se results in damage to the cells and consequently a low recovery rate with poor morphological preservation. The damage is caused by the tremendous kinetic energy, almost 7 million times that imparted by simple gravitational sedimentation, and the hydrostatic force of approximately 7 kg/cm² received by the cell even at a moderate centrifugation speed of 3500 rpm.2 Various modifications, such as the addition of serum, egg albumin, or formalin, and low temperature centrifugation have been introduced to improve the results, with little success.3,6,8,12

Simple gravitational sedimentation was de-
veloped to overcome the disadvantages of centrifugation. However, the methods recommended require a large volume of fluid, a somewhat complicated apparatus, and a long time period before recovery, resulting in cellular deterioration. Flocculation methods in which the cells are entrapped in a precipitate are unsatisfactory because of laborious preparation methods and cellular distortion. Filtration using the multiple pore cellulose filter (Millipore)* provided a very effective method of concentrating cells in the CSF, but several important factors have precluded its widespread use: 1) rapid clogging of the filter by blood and protein, 2) sensitivity of the filter to many solutions conventionally used in fixation and staining, 3) a tendency for the cells to become partially hidden in the filter substance, and 4) cellular deformation secondary to adhesions between the cells and the filter as well as the pressure often required to achieve rapid filtration.

Recent fabrication of a membrane filter that overcomes most of these problems has allowed the development of a bedside membrane filter technique for CSF cytology. A 3-year evaluation of the technique is presented.

Materials and Methods

A Nuclepore filter is the basis of the apparatus used in this technique. The filter is formed by bombarding a special thin plastic film with uranium fission fragments, producing damage tracts in the film. The plastic material is then subjected to an etching solution until the tracts are enlarged to the desired pore size. The resultant sieve is transparent and inert. A pore size of 8.0 μ was selected in order to allow a maximal filtration rate while retaining virtually all nucleated cells, the smallest of which should be lymphocytes ranging in size from 10–20 μ. The diameter of the filter is 25 mm, which allows concentration of the retained cells in a relatively small area and the mounting of the filter on a standard 1 × 3 inch glass slide, while at the same time permitting rapid filtration of cellular fluids. The filter is contained in a polyethylene filter holder and, when assembled, the apparatus is watertight (Fig. 1).

The recommended method of recovering and demonstrating cells in the cerebrospinal fluid is as follows.

Clinical Process

1. At least 10 mL of CSF is obtained at the time of lumbar puncture. Additional fluid will enhance the probability of detecting abnormal cells. After filtration, the fluid may be used for sugar, protein, or other determinations. If bacterial or fungal cultures are desired, a separate specimen should be obtained for this purpose.

2. A disposable 12 mL plastic syringe with the barrel removed, to serve as a funnel, is attached to the apparatus described above (Fig. 2).

3. The CSF is poured into the syringe and filtered immediately.

4. Before the CSF has totally drained from the syringe, the test tube containing the filtrate is replaced, and the apparatus with the contained cells is rinsed with a 5 mL aliquot of physiologic saline solution from a wash bottle.

5. Again, before the solution has totally drained from the syringe, the apparatus and cells are washed with 5 mL of 95% ethyl alcohol, which serves as a fixative.

6. Before the alcoholic solution has

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* Millipore Filter Corporation, Bedford, Massachusetts.
† General Electric Corporation, Pleasanton, California.
‡ Gelman Instrument Company, Ann Arbor, Michigan.
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drained completely from the syringe, the bottom plug is inserted into the filter holder, the syringe removed, and the top plug replaced. Thus, the filter with the recovered cells is left in an alcohol-filled container that can be kept until transfer to a laboratory for staining.

7. It is extremely important that positive or negative pressure not be applied to the fluid at any time, as this will cause cellular deformation and damage.

Laboratory Process

1. The filter is removed from the filter holder and held on a standard 1 × 3 inch glass slide by metal clips.
2. The cells are then stained in the standard fashion with the Papanicolaou-type stain and may be processed together with other cytologic preparations. If a hematogenous disease is suspected, a Wright’s stain should be used.
3. Generally, to avoid distraction during examination of the slide it is desirable after staining to clear the filter of the pores. To do this, the filter is turned cell side down, and the slide is kept wet with xylol, lifting and lowering the filter to remove trapped air. When the filter adheres to the slide without wrinkles or air spaces beneath, it is gently blotted dry with a paper towel. When dry, chloroform is distributed evenly over the surface of the filter from a pipette, dissolving enough of the filter to fill the pores and cause adherence to the slide. The slide is covered with a petri dish to allow slow evaporation of the chloroform. After the chloroform evaporates, the slide is again placed in xylol.
4. The slide is removed from the xylol, “Permount” mounting medium is added, and a cover slip is applied. The slide is then ready for examination.

Results

To determine the efficacy of this technique in cellular recovery and its effect on the filtered CSF, 50 CSF specimens were selected at random and one-half of each sample was filtered. Cell counts, as well as glucose and protein concentration determinations, were done on both the filtered and unfiltered specimens.

Cells

The prefiltration specimens contained from 0 to 320 nucleated cells per mm³ and 0 to 1450 erythrocytes per mm³, with the filtrate nucleated cell counts uniformly zero. By using an 8.0 μm pore size filter, most of the red blood cells, averaging 7.5 μm in diameter, will pass through, leaving the larger leukocytes and neoplastic cells. Rarely were any nucleated cells seen after filtration and, when seen, they were small, such as lymphocytes. The retained erythrocytes did not interfere with the evaluation of the slide. However, with bloody fluids, the red blood cells may be lysed with 1% acetic acid or Carnoy’s solution prior to filtration. The more cellular fluids tended to filter more slowly because of progressive plugging of the pores;
TABLE 1

Types of neoplasms detected by cytology

<table>
<thead>
<tr>
<th>Type of Neoplasm</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
<td></td>
</tr>
<tr>
<td>Malignant:</td>
<td></td>
</tr>
<tr>
<td>astrocytoma</td>
<td>3</td>
</tr>
<tr>
<td>meningeal sarcoma</td>
<td>2</td>
</tr>
<tr>
<td>atypical teratoma</td>
<td>1</td>
</tr>
<tr>
<td>medulloblastoma</td>
<td>1</td>
</tr>
<tr>
<td>reticulum cell sarcoma</td>
<td>2</td>
</tr>
<tr>
<td>Benign:</td>
<td></td>
</tr>
<tr>
<td>craniopharyngioma</td>
<td>2</td>
</tr>
<tr>
<td>ependymoma</td>
<td>1</td>
</tr>
<tr>
<td>chromophobe adenoma</td>
<td>1</td>
</tr>
<tr>
<td><strong>Metastatic</strong></td>
<td></td>
</tr>
<tr>
<td>lung</td>
<td>8</td>
</tr>
<tr>
<td>melanoma</td>
<td>4</td>
</tr>
<tr>
<td>urinary tract</td>
<td>2</td>
</tr>
<tr>
<td>breast</td>
<td>1</td>
</tr>
<tr>
<td>lymphocytic leukemia</td>
<td>1</td>
</tr>
<tr>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>30</td>
</tr>
</tbody>
</table>

however, this did not prove detrimental except in the loss of several additional minutes.

**Glucose**

Insignificant changes were seen in the glucose content of the post-filtered fluids. Usually no changes were found in the concentration of the sugar, and differences, when seen, were in the range of 1 to 2 mg%, the largest being 4 mg% in one specimen. Minimal elevation and lowering of the sugar content were seen with almost equal frequency and probably represent inherent errors in the technique of measurement.

**Protein**

The protein content of the filtered fluids, likewise, had clinically insignificant changes when compared to the unfiltered CSF. Sixty-eight percent of the CSF samples were within the normal range (45 mg% or less). None had changes greater than 4 mg%. Thirty-two percent of the samples had an abnormal protein content. Of the values below 200 mg%, no sample changed greater than 10 mg%, above 200 mg% the greatest change was 20 mg%. None of the abnormal values declined to the normal range after filtration, nor did any normal values become elevated to the abnormal range.

**Cytology**

The membrane filter technique described herein has been used in the cytologic examination of CSF obtained from approximately 80 patients undergoing lumbar puncture either for CSF evaluation or at the time of pneumoencephalography. Forty-one of these patients subsequently were found, either at operation or autopsy, to have neoplasms involving the brain or spinal cord. In 30 cases, abnormal cells were demonstrated on the filter, and 26 of these manifested cellular characteristics of malignancy (Table 1). Seventeen cases of metastatic disease were diagnosed, and in four of these, malignancy had not been previously suspected. Nine cases of malignancy were primary intracranial tumors. Benign tumors were suspected in four cases.

The cytologic examination was proven falsely negative in 11 cases. These cases represented a variety of tumors characterized for the most part as extradural in location or deep within cerebral tissue, not having a surface in contact with CSF. No false positive interpretations occurred. Thus, in the majority of patients with proven intracranial or intraspinal neoplasms located in a site where exfoliation of cells into the CSF was possi-

![Fig. 3. A group of cells displaying enlarged pale-staining nuclei and scanty, indistinct cytoplasm. The arrow indicates a slightly out of focus lymphocyte for size comparison. A malignant glioma of the right temporal lobe was demonstrated at operation. Papanicolaou stain, ×1755.](image-url)
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Fig. 4. A group of cells with pale-staining indistinct cytoplasm and elongated nuclei that are also pale-staining. Occasional nucleoli are present. Adjacent cells are out of focus. A suprasellar ependymoma was found at operation. Papanicolaou stain, ×1755.

Fig. 5. A large minocleated cell with quite sparse cytoplasm. The nuclei show some chromatin clumping and large nucleoli. Meningeal sarcoma was demonstrated at operation. Papanicolaou stain, ×2430.

Fig. 6. This very large multinucleated cell demonstrates hyperchromatic sparse cytoplasm. The nuclei are pleomorphic with prominent nucleoli. Adjacent inflammatory cells are seen. A metastatic bronchogenic carcinoma of the posterior fossa was demonstrated at operation. Papanicolaou stain, ×1890.

Discernible abnormal cells were demonstrated by means of this technique. Most important is the fact that in almost all cases the cellular morphology was extremely well preserved (Figs. 3–7).

Discussion

Cerebrospinal fluid cytology is valuable not only in the evaluation of patients with malignant intracranial and intraspinal tumors, but also with some benign neoplasms and non-neoplastic conditions. Unfortunately, lack of a suitable concentration technique has heretofore prevented full realization of the potential of this diagnostic tool. The technique described above most closely approaches the ideal and has many advantages over methods used in the past. It permits virtually total cell recovery from a given CSF specimen, yet is inexpensive, simple, and rapid. The filter is relatively inert and is unaffected by the commonly used fixatives and stains. The fluid is not lost and can be used for other determinations, since no clinically significant changes in the glucose or protein content occur with filtration. The cells are immediately fixed and preserved in the fixative solution contained in the watertight filter holder. Therefore, the time-dependent changes of cellular deterioration which begin shortly after CSF removal are avoided.17

It is not necessary to time the diagnostic work-up according to the availability of the cytologic laboratory. With the method described herein, the specimen may be sent at
FIG. 7. This group of cells demonstrates marked pleomorphism with rather scant densely staining cytoplasm. Occasional binucleated cells are seen. The nuclei are also very darkly staining with chromatin clumping. Occasional cells contained melanin pigment in their cytoplasm. Metastatic malignant melanoma. Papanicolaou stain, × 1890.

a later time to a general cytologic laboratory for preparation and evaluation. Once the filter is mounted on the standard glass slide, it can be processed in the same manner as any other exfoliative cytologic specimen, the only difference being the extra step of obliterating the pores before applying the cover slip. Because no force is required other than that offered by the hydrostatic force of the filtering column of CSF the cellular morphology is extremely well preserved.

It is not expected that the general cytologist will be able to substantiate a specific diagnosis without the experience of examining a number of CSF samples. However, identification of malignant cells should pose little difficulty, and often these cells can be related to either an epithelial or glial origin.

Acknowledgments

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References

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