Cytology of Cerebrospinal Fluid: Technical and Diagnosis Considerations

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ABSTRACT

The usefulness of cerebrospinal fluid cytology in the diagnosis of malignancy has been enhanced in recent years by the development of new techniques for increasing cellular yield. Micropore filters and a specially adapted cytocentrifuge have been used for this purpose with a resulting increase in cell concentration and in diagnostic accuracy. Cerebrospinal fluid examination has been found to be of particular value in the diagnosis of metastatic carcinoma, lymphomatous and leukemic involvement of the meninges and certain primary central nervous system tumors. Cytologic criteria for the differential diagnosis of the principal types of tumors encountered in cerebrospinal fluid are discussed.

Introduction

The application of cytologic detection of malignancy to the examination of cerebrospinal fluid has been well established over the past two decades and there have been several reviews on this subject. Nevertheless, the value of this procedure has been somewhat limited by the generally sparse cellularity of specimens prepared by standard methods and by the relative lack of experience of most laboratories in the cytologic evaluation of cerebrospinal fluid. In the past few years, refinements and modifications of technique have been introduced which provide increased cellular concentration and enable a more precise and easily understood classification of the types of malignant cells found. The purpose of the present report is to describe and compare the various techniques available for the cytologic examination of cerebrospinal fluid and to define the cytologic criteria for the diagnosis of different tumor types.

Techniques

The standard methods of cytologic evaluation of cerebrospinal fluid involve microscopic examination of the sediment obtained following centrifugation of the fluid. The specimens may be wet films stained with a dye such as toluidine blue or the more usual smears fixed in 95 percent alcohol and stained by the routine Papanicolaou technique. The wet-film preparations can be rapidly and easily prepared. Minimal cellular distortion is present. However, if cellularity is sparse as is the case in many CSF specimens, the number of cells present may be insufficient to establish a diagnosis even in the
case of clear-out malignancy. Furthermore, wet-film preparations are not permanent which is a major disadvantage. More generally used are the fixed Pap-stained smears which can be retained indefinitely. They do suffer from lack of sufficient cellular concentration and this has been a significant deterrent to the wider application of cerebrospinal fluid (CSF) cytology.

Because of this difficulty, newer methods have been developed to increase the cell recovery rate. These have been of two main types: (1) micropore filters for concentrating cells and (2) a special cytocentrifuge in which air-dried films can be prepared on a glass slide. These techniques have been described in detail elsewhere.3,8,11-12,14 The discussion here will be limited to a comparison of their usefulness in evaluation of CSF.

Two types of micropore filters have been used in the cytology laboratory, the Millipore filter* and the Nuclepore filter†. The Millipore technique, as employed in our laboratory, utilizes a 19 by 42 mm rectangular filter with pore size of 5 μm. Using a 1000 ml filtering flask and a suction device producing minimal negative pressure, the specimen is poured into the funnel so as to just cover the filter. After staining with a modified Papanicolaou method, the filter is mounted on a glass slide and a coverslip is added. Since the filter itself is stained, an opaque blue background is present. The cells are well concentrated on the filter and clearly defined. Slight distortion is present owing to shrinkage.

The Nuclepore is a later development which eliminates the opaque background. The filter is a disc which is one inch in diameter with 7 to 8 μm pore size. Pores are visible under the microscope with this type of filter, occasionally interfering with screening. This problem can be overcome by dissolving the filter with chloroform, but the use of this toxic chemical is somewhat hazardous in the cytology laboratory. Furthermore, comparison of the Millipore and Nuclepore filters has shown a significantly greater cell concentration with the Millipore technique.1

The cytocentrifuge‡ is of special value for the routine cell count and differential on CSF specimens. Specimens are placed in the chambers of the cytocentrifuge with glass slides in apposition to the exit ports of the chambers and centrifuged at 1200 rpm for 10 min. The slides are then air-dried and stained by the Wright-Giemsa method. The procedure is rapid and simple, requiring a minimum of instruction to use.4,14 Cellular concentration is greater than by routine centrifugation, but less than with filter techniques.1 A major drawback is the variable and sometimes considerable cellular distortion that is produced, particularly in nuclear appearance. While the filter techniques may produce slight cellular contraction, nuclear morphology is well preserved. The latter is, of course, a critical factor in the evaluation of malignancy.

In our experience, the Millipore filter provides consistently high cellular concentration and satisfactory cell preservation. Although somewhat time-consuming and requiring careful attention during processing, these are considered minor drawbacks in relation to the superior quality of preparations obtained. In comparison to cytocentrifuge specimens, the filters are much easier to interpret owing to better and more consistent cell preservation and are more likely to provide an accurate diagnosis owing to greater cell concentration.

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* Millipore Filter Corporation, Bedford, MA.  
† General Electric Company, Pleasanton, CA.  
Our general procedure in handling CSF specimens is to prepare both a routine Papanicolaou-stained fixed smear and a Millipore filter. A third Wright-stained smear is prepared if there is sufficient material or if there is a clinical indication of lymphoma or leukemia. The cellular characteristics of the latter lend themselves to examination with Wright’s stain. The Millipore filter is also quite satisfactory for the evaluation of these cell types.

The advantages and disadvantages of the different techniques are summarized in table I. A detailed comparison of the cell recovery rates with the filter and cytocentrifuge techniques can be found in a separate report.¹

### Diagnosis

The types of malignant tumors that can be diagnosed by CSF cytology fall into three major categories: (1) primary tumors of the central nervous system; (2) leukemias and lymphomas involving the central nervous system; and (3) metastatic carcinoma. These occur in a ratio of approximately 2:1:2, as shown in table II. The figures indicated are drawn from the review of Spriggs and Boddington,¹³ which included 72 cases where malignant cells were found. Comparable results have been observed in our material. Of 275 CSF specimens received in the University of California at San Francisco Cytology Laboratory over a 12 month period from July 1973 to June 1974, there were 26 in which malignant cells were present, including 11 with primary CNS tumors, 5 with leukemia or lymphoma, and 10 with metastatic carcinoma.

The presence of malignant cells in CSF is dependent on access of the tumor cells to the meninges and ventricular spaces. Thus, there are certain types of tumors that are likely to produce positive CSF findings. This is generally true of metastatic tumors and the lymphoma-leukemia group, which may become disseminated and involve the meninges. Among the primary CNS tumors, medulloblastoma, because of its invasive character, frequently produces positive findings, while gliomas and meningiomas are less likely to do so. Cells from tumors of glial origin can be found with some frequency in fluid removed at craniotomy, but this is less often the case in specimens obtained by lumbar puncture. Meningiomas, in spite of their location, rarely exfoliate cells into the cerebrospinal fluid owing to their restricted and delimited growth pattern.

In our material, the principal diagnostic problems encountered in the examination of CSF related to four tumor types: lymphoma and leukemia, medulloblastoma, astrocytoma and metastatic car-

### TABLE I

*Techniques for Cytologic Examination of Cerebrospinal Fluid*

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet-film (Toluidine blue)</td>
<td>Simple, rapid</td>
<td>Scant cellularity, non-permanent</td>
</tr>
<tr>
<td>Fixed smear (Pap stain)</td>
<td>Simple, good cell preservation</td>
<td>Scant cellularity</td>
</tr>
<tr>
<td>Fixed smear (Wright’s stain)</td>
<td>Useful in case of leukemia, lymphoma</td>
<td>Inadequate nuclear detail for other cell types</td>
</tr>
<tr>
<td>Millipore filter</td>
<td>Good cellular concentration</td>
<td>Cell distortion, complex, time-consuming</td>
</tr>
<tr>
<td>Nuclepore filter</td>
<td>Good cellular concentration</td>
<td>Cell distortion, complex, time-consuming</td>
</tr>
<tr>
<td>Cytocentrifuge</td>
<td>Rapid, simple</td>
<td>Poor cell preservation</td>
</tr>
</tbody>
</table>
Relative Frequency of Different Types of Tumors Producing Positive Cerebrospinal Fluid Cytology

<table>
<thead>
<tr>
<th>Type</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary central nervous system neoplasm</td>
<td>38</td>
</tr>
<tr>
<td>medulloblastoma, astrocytoma, ependymoma, meningioma, pineal teratoma</td>
<td></td>
</tr>
<tr>
<td>Leukemia and lymphoma</td>
<td>18</td>
</tr>
<tr>
<td>Metastatic carcinoma</td>
<td>44</td>
</tr>
<tr>
<td>lung, breast, kidney, stomach, colon</td>
<td></td>
</tr>
</tbody>
</table>

carcinoma. The distinguishing cytologic features of these tumors are summarized in Table III. Specific problems in differential diagnosis are described in detail.

Cells from lymphocytic lymphoma and leukemia must first of all be distinguished from circulating lymphocytes. The latter represent a normal component of cerebrospinal fluid and, in most Millipore preparations, scattered mature lymphocytes are present. In the presence of a malignant lymphoproliferative disorder, large numbers of lymphoid elements of varying degrees of maturity are found. The malignant cells have large nuclei, irregular nuclear outlines and prominent nucleoli (figure 1). Differentiation from medulloblastoma is an important consideration, since the two types of malignancy may occur in the same young age group and both are characterized by small round cells with hyperchromatic nuclei and scant cytoplasm. In the case of medulloblastoma, cell aggregates are present in which there is molding and superimposition of adjacent cells (figure 2), while in malignancies of lymphoid origin the cells are individually distributed without direct contact. Clinical history is also useful in distinguishing these two groups.

Astrocytoma cells must be differentiated from normal histiocytes and monocytes that are occasionally encountered in CSF, since these different cell types are similarly single and ovoid with eccentric nuclei. The cells from an astrocytoma differ in that their shape is more irregular, the nucleocytoplasmic ratio higher and other criteria of malignancy such as coarse chromatin clumping and nuclear hyperchromasia are evident (figure 3). Distinction from metastatic carcinoma can be a problem, since the latter may also occur as single cells. However, careful search will generally reveal at least a few groupings in metastatic carcinoma, and these are characterized by relatively little cytoplasm and pleomorphic appearance (figure 4). The most common primary sources of metastatic carcinoma are breast and lung. In the former case, the cells are small and round, with arrangement in linear or ball-like groupings, although occasionally large single cells can be seen. Metastatic lung tumors may show squamous differentiation, gland formation or undifferentiated appearance depending on the histologic type. History is helpful in such cases, but on occasion initial diagnosis may be made on the basis of CSF findings. The presence of cell groups with high nucleocytoplasmic ratios, nuclear hyperchromasia and prominent nucleoli points to a diagnosis of metastatic tumor. Cells from an ependymoma may present similar features, but the distinction can generally be made in terms of clinical findings.
Other types of primary CNS tumors which have been encountered in cerebrospinal fluid include meningioma, pineal teratoma and retinoblastoma. These have distinctive clinical and cytologic features and, in the presence of an adequate cellular sample and clinical history, do not present a problem in differential diagnosis.

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References