Cytologic Characteristics of Meningeal Carcinomatosis

Increased Diagnostic Accuracy Using Carcinoembryonic Antigen and Epithelial Membrane Antigen Immunocytochemistry

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Background and Objectives: Traditionally, the diagnosis of meningeal carcinomatosis has been based on clinical suspicion and confirmed by cytologic study of cerebrospinal fluid. However, routine cytologic study may fail to detect malignant cells in a relatively large number of cases. We used immunocytochemistry in an attempt to increase the sensitivity of cytologic detection of malignant neoplasms in cerebrospinal fluid.

Materials and Methods: Thirty-eight consecutive cerebrospinal fluid specimens from patients with clinically suspected meningeal carcinomatosis were selected for this study. Immunocytochemistry for carcinoembryonic antigen and epithelial membrane antigen were used on the archival Papanicolaou-stained cerebrospinal fluid preparations.

Results: Of the 23 specimens from patients with proven meningeal carcinomatosis, 13 were correctly diagnosed using cytomorphologic criteria alone. The diagnosis of malignant neoplasm in 8 cytologically suspicious and 1 cytologically negative specimen was confirmed using immunocytochemistry. All cases that were negative on follow-up were also negative cytologically and immunocytochemically.

Conclusions: We conclude that in using common antibodies, such as carcinoembryonic antigen and epithelial membrane antigen, the sensitivity of the cytologic diagnosis of meningeal carcinomatosis increases, and that previously Papanicolaou-stained preparations are suitable for immunocytochemical studies.

Arch Neurol. 1998;55:181-184

N infiltration of the leptomeninges by malignant cells is an important neurologic complication that occurs in 5% to 18% of patients with solid tumors.1 In the breast and lung are the most frequent sources of metastasis.2,3 Since a favorable clinical course depends largely on early diagnosis and therapy, an accurate and rapid diagnostic method is essential. Traditionally, the diagnosis of meningeal carcinomatosis has been based on clinical suspicion confirmed by cytologic study of cerebrospinal fluid (CSF).4 However, routine cytologic study may fail to detect malignant neoplasms in a relatively large number of cases because of the limited number of carcinoma cells in CSF showing minimal pleomorphism and frequent association with reactive pia-arachnoid mesothelial (PAM) and inflammatory cells.2,6 Repeated lumbar punctures are shown to increase the diagnostic yield of CSF cytologic studies; however, the procedure may be associated with increased morbidity and patient discomfort.

Immunocytochemical markers have been widely used to facilitate detection and subclassification of carcinoma cells in cytologic material.5,9 In an attempt to increase the sensitivity of cytologic detection of malignant neoplasms in CSF, we used immunocytochemistry for carcinoembryonic antigen (CEA) and epithelial membrane antigen (EMA) in archival Papanicolaou-stained preparations.

RESULTS

All 4 Papanicolaou preparations in each case were reexamined first, and the diagnoses of malignant neoplasm, suspicious for malignancy, and negative were confirmed using cytomorphologic criteria. After immunostaining, the slides were reviewed without knowledge of the previous diagnoses. Positive results for CEA and EMA were defined as finely granular, brown cytoplasmic, and/or membranous granular staining. Neutrophils, when present, reacted positively with the polyclonal CEA antibody and were used as...
MATERIALS AND METHODS

Thirty-eight consecutive CSF specimens from 38 patients with clinically suspected meningeal carcinomatosis were selected from cytology files at the University of Miami/Jackson Memorial Medical Center, Miami, Fla. Cerebrospinal fluid specimens that were diagnosed as malignant, suspicious for malignancy, or negative were included in this study. The diagnosis of malignant neoplasm was rendered when definitive malignant epithelial cells, isolated or in abnormal groups, were observed. When few atypical cells were seen in a hypocellular sample or in the presence of bland cytologic features not conclusive for the diagnosis of carcinoma, the case was diagnosed as being suspicious for malignancy. A negative diagnosis was rendered in samples with few benign lymphocytes and/or reactive PAM cells. These cells were identified as normochromatic large cells with a normal nucleocytoplasmic ratio showing occasional grouping. Acellular specimens and those representing lymphoma, leukemia, and primary brain tumors were excluded from the study.

Cases that were positive on clinical follow-up included patients with aseptic meningitis with laboratory correlation, along with abnormal results from a computed tomographic scan of the head and no improvement of the patient’s condition despite treatment.

Slides of 4 cytocentrifuged smears that were fixed in 95% ethanol and stained by the Papanicolaou method were available in each case.

The coverglasses of 2 Papanicolaou-stained slides were removed by placing them in xylene for approximately 1 to 4 hours. The slides were then hydrated in decreasing grades of alcohol. Endogenous peroxidase activity was blocked by immersing the slides in 3% hydrogen peroxidase in methanol for 20 minutes. The slides were then treated with normal horse serum for 20 minutes. Polyclonal rabbit antiserum against human CEA (code A115, Dako, Carpinteria, Calif) was used at a dilution of 1:200 for 30 minutes at room temperature. Anti-rabbit immunoglobulin (PK-4001, Vector, Burlingame, Calif) was used at a 1:600 dilution. Monoclonal mouse antibody against human EMA (code M613, Dako) diluted at 1:600 for 30 minutes at room temperature was followed by antimouse immunoglobulin (PK-4002, Vector) at a 1:600 and avidin-biotin horseradish peroxidase complex at a 1:1600 dilution. The slides were washed in 3 changes of phosphate-buffered saline solution between steps. Diaminobenzidine was used as the chromogen. Counterstaining was not needed. The slides were then rinsed in tap water and dehydrated in increasing grades of isopro pyl alcohol, cleared with xylene, and mounted using a synthetic neutral resin.

Cytologic examination of CSF has been accepted as a routine diagnostic technique in patients with known or suspected malignant neoplasms involving the central nervous system. Among metastatic solid tumors involving the leptomeninges, carcinomas of the lung and breast most commonly shed cells in CSF.1-5 Carcinoma cells in general have a tendency to occur singly or in small, loose clusters in CSF. This is in contrast to the cell balls or large cohesive sheets seen in fluids of other body cavities. The relatively bland morphologic features of the isolated carcinoma cells in CSF can create diagnostic difficulty. The presence of large, reactive PAM cells in CSF may add to the difficulty of detecting carcinoma cells as well. This may result in rendering an inconclusive diagnosis that is of little value in the management of a patient with clinically suspected meningeal carcinomatosis. Such reports are usually followed by repeated lumbar punctures, a procedure that can be associated with complications.

Immunocytochemistry has been used to improve the sensitivity and specificity of cytologic diagnosis in CSF.5,10

Table 1. Cytologic Diagnoses, CEA and EMA Immunostainings, and Outcome in 38 CSF Specimens*

<table>
<thead>
<tr>
<th>Cytologic Diagnosis</th>
<th>No. of Cases</th>
<th>CEA</th>
<th>EMA</th>
<th>CEA and EMA</th>
<th>No. of Cases Positive on Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>13</td>
<td>5</td>
<td>9</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>Suspicious for malignant neoplasm</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Negative</td>
<td>16</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>12</td>
<td>15</td>
<td>8</td>
<td>23</td>
</tr>
</tbody>
</table>

*CEA indicates carcinoembryonic antigen; EMA, epithelial membrane antigen; and CSF, cerebrospinal fluid.

The technique is particularly useful in highlighting rare malignant cells within an inflammatory-reactive background. However, not everyone agrees that immunocytochemistry increases the sensitivity of cytologic diagnosis of malignant neoplasm in CSF. The purpose of this study was to assess the value of immunocytochemical staining for CEA and EMA in the differential diagnosis of reactive PAM cells and carcinoma cells in CSF. Our results show that positive immunostaining for CEA and EMA is highly sensitive and specific for the diagnosis of carcinoma in CSF.

Simultaneous use of both antigens improved the sensitivity of our cytologic diagnosis from 57% to 83% (Table 2).

Most common metastatic carcinomas in the central nervous system, such as those from the breast, lung, and gastrointestinal tract, are usually positive for CEA and/or EMA. A number of other carcinomas, such as serous ovarian tumors, hepatocellular carcinomas, renal cell carcinomas, and prostatic carcinomas, are usually negative for CEA; however, these tumors are rarely found in CSF and are not usually considered in the differential diagnosis. Moreover, most of these tumors are at least focally positive for EMA. On the other hand, PAM cells are known to be negative for EMA and CEA.

In our study, the majority of carcinomas originated from the lung or breast (Table 3). Fifty-two percent stained positively for CEA and 65% for EMA. Eighty-three percent of cases were positive for CEA and/or EMA. Using immunostaining for EMA, we confirmed the diagnosis of malignant neoplasm in 6 of 8 cytologically suspicious cases. Using CEA in the same cases, we were able to confirm the diagnosis of carcinoma in all of them. Although the primary tumor site was not evident in 4 of the 23 patients with meningeal carcinomatosis, immunocytochemistry was helpful in reaching the definitive diagnosis of cancer in 2 of those cases. In 4 patients (17%) with clinically proven carcinoma, neither cytomorphologic studies nor immunocytochemical studies for CEA and EMA could detect malignant cells in CSF. These represent clinically false-negative cases resulting perhaps from the lack of shedding of tumor cells.

In summary, although immunocytochemistry cannot replace conventional cytologic analysis of CSF, it increases the sensitivity of the diagnosis of meningal carcinoma. The use of CEA and EMA together increases the sensitivity more than CEA or EMA alone. Finally, previous alcohol-fixed Papanicolaou-stained cytocentrifuge specimens are suitable for immunocytochemical localization of these antigens. We therefore recommend the

![Figure 1. Proven carcinoma cells in cerebrospinal fluid. Primary site was the lung; positive immunoperoxidase staining for carcinoembryonic antigen (original magnification ×250).](image1)

![Figure 2. Proven carcinoma cells in cerebrospinal fluid. Primary site was the breast; positive immunoperoxidase staining for epithelial membrane antigen (original magnification ×250).](image2)

| Table 2. Statistical Results of Routine CSF Cytologic Studies Before and After Applying Immunocytochemistry |
|---|---|---|
| Result | Cytology Alone | Cytology and Immunocytochemistry |
| True positive | 13 | 19 |
| False positive | 0 | 0 |
| True negative | 15 | 15 |
| False negative | 10 | 4 |
| Sensitivity, % | 57 | 83 |

| Table 3. Type of Malignant Neoplasm Found and Immunoperoxidase Results Seen in Cerebrospinal Fluid Specimens* |
|---|---|---|
| Type of Malignant Neoplasm | CEA and/or EMA Positive | CEA and EMA Negative |
| Breast carcinoma | 11 | 1 |
| Lung carcinoma | 6 | 1 |
| Unknown origin | 2 | 2 |
| Total | 19 | 4 |

* CEA indicates carcinoembryonic antigen; EMA, epithelial membrane antigen.
use of these 2 markers in Papanicolaou-stained CSF specimens that are suspicious for malignancy, particularly those from patients with a history of carcinoma.

Accepted for publication July 7, 1997.

We thank Estela Garcia-McDougal, MPA, SCT (ASCP), for her assistance in preparation of the manuscript.

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REFERENCES


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