The value of interphase cytogenetics in cytology for the diagnosis of leptomeningeal metastases

Article abstract—We studied the use of fluorescence in situ hybridization (FISH) in CSF to enhance the diagnostic yield for the detection of malignancy on the first lumbar puncture in patients clinically suspected of having leptomeningeal metastases (LMM). Although repeated lumbar punctures were still needed in some patients, the use of FISH did speed up the diagnosis in approximately one-third of the patients clinically suspected of having LMM with atypical cells at first cytology. This eliminates the need for repeated lumbar punctures in these patients and enables an earlier start of treatment.

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The detection of malignant cells in CSF on cytologic examination is generally required for a definitive diagnosis of leptomeningeal metastases (LMM). However, cytodiagnosis of CSF using conventional staining procedures fails to detect malignant cells in up to 15% of clinically suspected cases, even after repetitive lumbar punctures. None of the additional tests, such as immunochemistry, biochemical tumor marker assays, and cytogenetic approaches, significantly improved the diagnostic accuracy because of their lack of sensitivity. The detection of aneuvery by fluorescence in situ hybridization (FISH) in various body fluids other than CSF has proved to be as sensitive as conventional cytodiagnostic procedures and is recommended as a complement to routine cytologic examination. Recently, we indicated that malignant cells can be detected in CSF by demonstrating aneuvery for chromosome 1 using FISH.

The aim of this study was to establish the additional diagnostic value of FISH in the cytologic examination of the first CSF sample of patients clinically suspected of having LMM.

Methods. CSF obtained at the first lumbar puncture from patients clinically suspected of having LMM was used
Table: Combined results of cytology and fluorescence in situ hybridization (FISH) in the first CSF sample of 45 patients clinically suspected of having leptomeningeal metastases

<table>
<thead>
<tr>
<th>Result of cytology</th>
<th>Result of FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant cells</td>
<td>17/27</td>
</tr>
<tr>
<td></td>
<td>NCAs present</td>
</tr>
<tr>
<td></td>
<td>7/27</td>
</tr>
<tr>
<td></td>
<td>NCAs absent</td>
</tr>
<tr>
<td></td>
<td>Not done/not assessable 3/27</td>
</tr>
<tr>
<td>Atypical cells</td>
<td>3/8</td>
</tr>
<tr>
<td></td>
<td>NCAs present</td>
</tr>
<tr>
<td></td>
<td>5/8</td>
</tr>
<tr>
<td></td>
<td>NCAs absent</td>
</tr>
<tr>
<td>Normal cells</td>
<td>6/10</td>
</tr>
<tr>
<td></td>
<td>NCAs present</td>
</tr>
<tr>
<td></td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>NCAs absent</td>
</tr>
</tbody>
</table>

NCAs = numerical chromosomal aberrations.

both for cytology and interphase cytogenetics. FISH was essentially done as described before after an enzymatic pretreatment step using pepsin, the biotinylated DNA probe for chromosome 1 (1q12, pUC 1.77, Cooke and Hindley*) was hybridized overnight. After hybridization, the slides were washed under stringent conditions, avoiding minor bindings. The probe was detected using two layers of fluorescein-isothiocyanate conjugated to avidin coupled by a layer of biotinylated goat anti-avidin. The slides were evaluated using previously described criteria.

The results obtained with FISH were compared with those of the cytologic evaluation of Giemsa stained preparations. Also, the results of FISH were correlated to the cell number of CSF and to the type of primary tumor. LMM was diagnosed when malignant cells were demonstrated by cytology or when malignant leptomeningeal infiltration was detected at autopsy.

Results. The CSF of 45 patients was cytologically examined because of a clinical suspicion of LMM of the following malignancies: breast carcinoma (15), non-Hodgkin lymphoma (9), small-cell lung carcinoma (5), non—small-cell lung carcinoma (4), acute lymphoblastic leukemia (4), adenocarcinoma of unknown origin (3), renal cell carcinoma (2), medulloblastoma (1), dyggerminoma (1), and anaplastic bladder carcinoma (1). Eventually, LMM was diagnosed in 34 of these patients. The table shows the results obtained by cytology and FISH of the first CSF sample.

The first cytologic examination of the 45 samples classified 10 cases as normal, 27 as malignant, and 8 as atypical. Forty-two of the 45 samples could be evaluated by FISH. Three malignant samples were not assessable because of interpretation difficulties due to lysis or absence of cells on the slides.

Only disomic cells were detected by FISH in the 10 samples of the patients with normal cytologic results, whereas in 17 of the 24 assessable malignant samples (71%), numerical chromosomal aberrations (NCAs) for chromosome 1 were detected in 5 to 28% of the nuclei. FISH clearly detected NCAs in three of the eight (38%) samples with atypical cells at first cytologic examination. In all three cases, malignant cells were cytologically demonstrated after repetitive lumbar punctures. Thus, in approximately one-third of the patients with atypical cells at first cytologic examination, multiple lumbar punctures could have been avoided.

Positive FISH results were found in 74% (17 of 23) of the solid tumors with proven LMM and in 27% (3 of 11) of the lymphoproliferative diseases with proven LMM. Furthermore, NCAs were more often detected in malignant samples with a high cell number (above 3 cells per μm) (81%) than they were in those with a low cell number (50%). However, two of the three atypical samples with NCAs detected by FISH had a low cell number.

The FISH results in the patients ultimately diagnosed as not having LMM showed no aberrant cells. Although the specificity of FISH was 100%, the negative predictive value was only 50%.

After repeated cytologic examination, five of the eight samples with atypical cells were classified as malignant, increasing the sensitivity of cytology to 94%, but an extra 20 lumbar punctures in all had to be done for this. One more case with atypical cells could be classified as LMM at autopsy. The 10 samples classified as normal at first cytologic examination remained normal even after three lumbar punctures. However, LMM was demonstrated at autopsy in one of these cases.

Discussion. Detection of malignant cells in the CSF by conventional cytology is generally required to establish the diagnosis of LMM, although this approach is only moderately sensitive. The aim of this study was to analyze the additional diagnostic value of FISH by detecting aneuxyomy for chromosome 1 in the cells present in the CSF of patients clinically suspected of having LMM to demonstrate malignancy, especially if the first cytologic examination was inconclusive. Although this chromosome generally detects aneuxyomy rather than being tumor-specific, chromosome 1 is frequently involved in the carcinogenesis of solid tumors and, to a lesser extent, during the progression of lymphoproliferative diseases. Furthermore, aneuxyomy for chromosome 1 in cells present in the CSF was only detected in malignant samples.

None of the cytologically normal samples displayed an NCA for chromosome 1. All 10 patients had two more lumbar punctures, which did not alter the first cytologic classification. One might argue that the finding of normal cytology combined with a normal FISH result in the first CSF sample is sufficient to exclude LMM. However, although the specificity of FISH was very good, a negative FISH result is not of additional diagnostic value due to its low negative predictive value. Furthermore, LMM was demonstrated at autopsy in one patient with persistently negative cytology and normal FISH results. Possibly, the adherence of the malignant cells to the leptomeninges was the strong that no cells were exfoliated into the CSF. In 71% of the samples classified as malignant at first cytology, nuclei with NCAs were detected by FISH. Although we could not classify all the cytologically malignant samples as aberrant, the sensitivity of FISH can be increased by using multiple centromeric DNA probes or by using specific DNA probes in cases with known genetic aberration of the primary tumor. In any case, FISH is not of additional value for a diagnosis of LMM in the case of positive cytology.
Of greater clinical importance is the finding of NCAs in three of the eight samples with atypical cells at first cytologic examination that proved to be malignant after repeated lumbar punctures. If FISH had been used as a diagnostic adjunct to the cytologic evaluation, extra lumbar punctures could have been avoided in approximately one-third of the clinically suspected patients with atypical cells in their CSF. Furthermore, the time delay before starting the treatment in this patient group could have been avoided, which might have influenced the clinical course, because this depends on early treatment.

Other cytogenetic techniques to classify atypical cells found in CSF have been studied before, such as flow cytometry (FCM), DNA single-cell cytometry, and PCR. However, none of these techniques can be used on a routine basis because either the specificity is too low or the method has shortcomings. For PCR, for example, the cytogenetic aberration must be known, and FCM requires many cells.

We have demonstrated an additional diagnostic value for FISH in classifying cytologically atypical cells in the CSF of patients clinically suspected of having LMM. NCAs detected by FISH obviated repetitive lumbar punctures in approximately one-third of these patients, resulting in a more rapidly definitive diagnosis. This might influence the clinical course through an earlier start of treatment.

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References