Biochemical Markers of Central Nervous System Tumors in Cerebrospinal Fluid*

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ABSTRACT

Central nervous system (CNS) tumor markers that have been measured in cerebrospinal fluid (CSF) include among others, lactate dehydrogenase (LDH), glutamic oxalacetic transaminase (GOT), aldolase, phosphohexose isomerase (PHI), lysozyme, creatinine kinase, isocitrate dehydrogenase, adenylate kinase, the polyamines and desmosterol. Such markers have at least five possible functions in the diagnosis and management of CNS neoplasms: screening, diagnosis, classification, early prediction of changes in clinical status and evaluation of efficacy of therapy. The predictive value model of Galen and Gambino defines four parameters (sensitivity, specificity, predictive value and efficiency) which can be used to determine the potential utility of each marker for each possible function.

Since the low prevalence of CNS tumors (4.5 cases per 100,000 individuals) makes screening in the general population impractical, this model has been applied to the use of the aforementioned markers for diagnosis of CNS tumors in patients with neurological symptoms. The data from previous clinical studies have been collated and used to calculate the sensitivity and specificity of each marker. Very little data are available relative to the potential utility of these markers for the last three functions mentioned, except for desmosterol and polyamines. Finally, the possibility of a diagnostic test for CNS tumors based on measurement of more than one marker is proposed as a means of improving the low specificity of many of the markers studied.

Introduction

Biochemical tumor markers have been defined as any substance whose presence, or whose elevation above normal concentrations, correlates with the presence of a tumor. Such tumor markers are produced in tumor tissue and released into the various physiological fluids, where they can be detected and quantitated. Measurements of tumor markers have five potential uses in the diagnosis and management of patients with central nervous system (CNS) neoplasms: (1) in screening...
the general population for presence of CNS tumors, (2) as a diagnostic tool in establishing presence or absence of CNS neoplasms in patients suspected of harboring tumors, (3) as an aid to histologic examination of biopsy specimens in classifying and grading CNS tumors, (4) as aids in early detection of changes in patient's clinical status subsequent to tumor regrowth and (5) in short term evaluation of the efficacy of therapy.

Galen and Gambino have presented four parameters for evaluating the utility of clinical laboratory tests in diagnosis and screening. These are: (1) sensitivity—the fraction of test subjects with the disease that give positive test results, (2) specificity—the fraction of test subjects free of the disease that give negative test results, (3) predictive value—the fraction of subjects giving positive test results that do have the disease and (4) efficiency—the fraction of all test results obtained that are correct predictions of presence or absence of the disease. While sensitivity and specificity can be calculated directly from the data obtained in a clinical study, predictive value and efficiency are dependent upon the prevalence of the disease in the population to which the diagnostic test is applied.

The prevalence of CNS tumors in the general population has been estimated as 4.5 cases per 100,000 individuals. Using this estimated prevalence and assumed values for sensitivity and specificity, predictive value and efficiency can be calculated in the following manner. An arbitrarily chosen total number of subjects is multiplied by the prevalence of CNS tumors to find the total number of subjects with tumors. The remainder of the total subject pool is free of tumors. The number of subjects with tumors giving positive test results is obtained as the product of sensitivity times the total number of subjects with tumor. The remainder of the total tumor-bearing patients would give negative results. The number of subjects free of tumors giving negative test results is found as the product of specificity times the total number of subjects without tumor; the remainder of the tumor-free patients would give positive results. Predictive value is the quotient of true positives divided by the total number of subjects giving positive results. Efficiency is the quotient of (true positives + true negatives) divided by the total number of subjects.

A hypothetical, near-perfect screening test for CNS tumors, with sensitivity and specificity both equal to 99 percent, would thus have a predictive value of only 0.44 percent as shown in table I. This means that only four of every 1,000 subjects giving positive test results would really have a CNS tumor. Screening for brain tumors is probably undesirable, unless a test with sensitivity and specificity both equal to 100 percent were available. Such a test could possibly be based on demonstrating the presence or absence of some tumor-specific antigen.

A diagnostic test for CNS tumors, in contrast to a screening test, would be applied to those neurological patients suspected of harboring CNS tumors. Since the prevalence of CNS neoplasms in this test population is so much higher than in the general population, those markers of CNS tumors that showed reasonably high sensitivity and specificity would have acceptable predictive values. Determination of the specificity of such markers, however, must not include data obtained from normal, healthy individuals since such subjects would not be included in the population to be tested.

Survey of Markers Used in Diagnosis

In the last 20 to 25 years, a great many clinical studies have been reported in which cerebrospinal fluid (CSF) levels of various molecules or activities of various enzymes have been measured in a wide variety of neurological patients. Some of
these, such as lactate dehydrogenase (LDH) or the polyamines, are enzymes or small molecules whose intracellular activity or concentration has been shown to be greater in neoplastic cells than in normal cells from the same tissue. Others, such as lysozyme, have been studied in spite of the fact that no real rationale exists for expecting them to be good CSF markers of CNS tumors.

Data have been collated from a large number of clinical studies in which levels of biochemical markers of CNS tumors have been measured in the CSF of patients with CNS neoplasms and other neurologic disease. Only those studies that reported their data as individual patient values, or as number of test subjects above and below a defined range for a reference group, were included in this compilation. The list of markers that have been studied, along with the number of subjects in each of four categories, are shown in table II. For each marker, a positive result was considered as greater than the mean plus twice the standard deviation for a reference group of individuals with no neurologic disease. A negative result was less than this level for the marker in question. Within each marker, the reference group used was that indicated by the authors of each study from which data were collated. True positives were patients with histologically verified tumors who had such elevations. False positives were patients with histologically verified tumors who had such elevations.

| TABLE I
| Calculation of Predictive Value and Efficiency for a Hypothetical Screening Test for Central Nervous System Tumors
<table>
<thead>
<tr>
<th># Subjects with Positive Test Results</th>
<th># Subjects with Negative Test Results</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td># Subjects with CNS tumors</td>
<td>4.45</td>
<td>0.05</td>
</tr>
<tr>
<td># Subjects without CNS tumors</td>
<td>999.95</td>
<td>98,995.55</td>
</tr>
<tr>
<td>Totals</td>
<td>1,004.4</td>
<td>99,995.6</td>
</tr>
</tbody>
</table>

Sensitivity = 99 percent
Specificity = 99 percent
Prevalence = 4.5 per 100,000
Predictive value = 4.45 per 1,004.4 = 0.44 percent
Efficiency = (98,995.55 + 4.45) per 100,000 = 99 percent

| TABLE II
| Results Collated from Clinical Studies of Cerebrospinal Fluid Levels of Biochemical Markers of Central Nervous System Tumors
<table>
<thead>
<tr>
<th>Individuals with CNS Tumors</th>
<th>Individuals with Neurologic Disease Other Than Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marker</td>
<td>True Positives</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>223</td>
</tr>
<tr>
<td>Glutamic-oxalacetic transaminase</td>
<td>115</td>
</tr>
<tr>
<td>Aldolase</td>
<td>21</td>
</tr>
<tr>
<td>Phosphohexose isomerase</td>
<td>39</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>29</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>21</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>66</td>
</tr>
<tr>
<td>Adenylate kinase</td>
<td>9</td>
</tr>
<tr>
<td>Putrescine</td>
<td>42</td>
</tr>
<tr>
<td>Spermidine</td>
<td>39</td>
</tr>
<tr>
<td>Combined polyamines</td>
<td>51</td>
</tr>
<tr>
<td>Desmosterol</td>
<td>115</td>
</tr>
</tbody>
</table>
negatives were patients with histologically verified tumors without elevated marker levels. True negatives are patients free of tumor who are also without elevated marker levels, while false positives are patients free of tumor with elevated marker levels.

In table III are listed the sensitivity and specificity calculated for each marker from the data in table II. Sensitivity was calculated as the quotient of true positives divided by (true positives + false negatives) times 100 percent. Specificity was calculated as the quotient of true negatives divided by (true negatives + false positives) times 100 percent. The data used in these calculations are from the results collated for each marker in table II. Since the prevalence of CNS tumors in the population of neurological patients is unknown, a figure of 35 percent has been used as an estimate for the prevalence of CNS tumors in the total subject pool given in table II. The predictive value and efficiency of each marker is also listed in table III. These parameters were calculated by the methods described in the discussion of table I.

### TABLE III

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive Value</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate dehydrogenase</td>
<td>66</td>
<td>62.5</td>
<td>48.5</td>
<td>63.7</td>
</tr>
<tr>
<td>Glutamic-oxalacetic transaminase</td>
<td>41</td>
<td>69</td>
<td>41.5</td>
<td>59.2</td>
</tr>
<tr>
<td>Aldolase</td>
<td>53.8</td>
<td>94</td>
<td>82.8</td>
<td>80</td>
</tr>
<tr>
<td>Phosphohexose isomerase</td>
<td>58.2</td>
<td>67.8</td>
<td>49.2</td>
<td>64.4</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>39.7</td>
<td>74.2</td>
<td>45.2</td>
<td>62.2</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>55.3</td>
<td>62.2</td>
<td>44</td>
<td>59.8</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>86.8</td>
<td>50</td>
<td>48.2</td>
<td>62.8</td>
</tr>
<tr>
<td>Adenylate kinase</td>
<td>81.8</td>
<td>57.7</td>
<td>50.9</td>
<td>66.1</td>
</tr>
<tr>
<td>Putrescine</td>
<td>66.7</td>
<td>75.7</td>
<td>59.5</td>
<td>72.6</td>
</tr>
<tr>
<td>Spermidine</td>
<td>61.9</td>
<td>77</td>
<td>59.1</td>
<td>71.7</td>
</tr>
<tr>
<td>Combined polyamines</td>
<td>81</td>
<td>66.2</td>
<td>56.2</td>
<td>71.4</td>
</tr>
<tr>
<td>Desmosterol</td>
<td>72.3</td>
<td>98.9</td>
<td>97.2</td>
<td>89.6</td>
</tr>
</tbody>
</table>

Isocitrate dehydrogenase is the marker with highest sensitivity (86.8 percent) for the detection of CNS tumors, although the specificity of this enzyme is a rather poor 50 percent. The desmosterol test appears to have the highest specificity (98.9 percent) and thus the best predictive value (97.2 percent) and efficiency (89.6 percent). This promising result is tempered by the limited range of non-neoplastic neurologic diseases that have been investigated with the desmosterol test. A second problem with this marker is that its use is dependent upon daily administration of 500 mg of triparanol (an inhibitor of conversion of desmosterol to cholesterol) for five days prior to lumbar puncture. The administration of triparanol to humans has been restricted in this country owing to toxic effects observed in high-dosage, long-term therapy. Of those markers for which both an adequate number of patients with CNS tumors and a more complete spectrum of non-tumor patients have been seen, it appears that the combined polyamine test may represent a good overall compromise for high sensitivity with reasonable specificity. Adenylate kinase is also a potentially useful marker, but it requires further study in a larger group of tumor and non-tumor patients.

#### Other Uses of Markers

Several of the markers listed in table II have been studied as potential aids to histologic examination of biopsy specimens in classifying and grading CNS tumors. While most studies of lactate dehydrogenase (LDH) isoenzyme distribution as an index of tumor malignancy have been done on tumor tissue homogenates,16,20,45,52,54 four reports of LDH isoenzyme distributions in CSF of brain tumor patients have appeared in the literature.15,23,46,43 Two of these studies observed decreases in the LDH-1 fraction and increases in the LDH-4 and LDH-5
fractions to be associated with malignant CNS tumors, while benign tumors had isoenzyme distributions no different from non-tumor controls.\textsuperscript{23,43} The other two studies found no difference in CSF LDH isoenzyme distributions between patients with benign and malignant CNS tumors. Several of the studies of CSF levels of LDH,\textsuperscript{19} glutamic oxalacetic transaminase (GOT),\textsuperscript{6,35} phosphohexose isomerase (PHI)\textsuperscript{44} and adenylate kinase\textsuperscript{42} reported elevated levels of enzyme activity to be associated with malignant CNS tumors but not with benign tumors.

In our own laboratories, elevated CSF levels of polyamines were found in all 33 cases of untreated medulloblastoma or malignant glioma investigated, while only 18 of 30 patients with benign tumors had such elevations.\textsuperscript{31} Although Fumagalli and Paoletti\textsuperscript{11} reported that a correlation does exist between CSF desmosterol levels and the degree of malignancy of the tumor, the greatest elevations they observed were in cases of meningioma and acoustic neurinoma. It appears, therefore, that none of the markers discussed in this paper can yet be considered as a reliable replacement for, or even as an adjunct to, histologic examination of biopsied tumor tissue.

Only two of the markers listed in table II, the polyamines and desmosterol, have been investigated as predictors of changes in clinical status of patients with CNS tumors. In our laboratories, serial determinations of CSF polyamine levels have been used by us to monitor patients being treated for brain tumors. It appears that, particularly in case of medulloblastoma, increases in CSF polyamine levels can precede radiographic or clinical evidence of tumor regrowth by weeks to months. Fumagalli et al\textsuperscript{12} investigated the desmosterol test as a predictor of tumor regrowth following surgical removal and reported that 22 of 29 patients with recurrent tumors gave positive test results while all seven patients who did not have recurrences had negative test results. The desmosterol test correctly predicted tumor regrowth in nine of 11 cases of glioblastoma, all three cases of ependimoma, both cases of medulloblastoma, both cases of meningioma, and both cases of pituitary adenoma examined. It failed in three of seven cases of Grade II astrocytoma and in one case each of craniopharyngioma and papilloma of the choroid plexus tested.

The polyamines and desmosterol are also the only markers that have been investigated as predictors of the efficacy of chemo- or radiotherapy of CNS tumors. A selected group of patients with brain tumors were followed with multiple lumbar punctures during the first week of therapy. Significant increases in CSF putrescine levels were observed four to six days after treatment in those patients whose tumors responded to therapy.\textsuperscript{31,32,33} Fumagalli et al\textsuperscript{13} studied changes in CSF levels of desmosterol for 11 patients being treated for brain tumors with nitrosourea compounds. While a number of changes in CSF desmosterol levels were observed, the authors do not indicate what changes in clinical status or response of the tumor to therapy accompanied the changes in sterol levels.

The possibility of establishing a diagnostic test for CNS tumors based on measurement of more than one marker in each CSF sample is worthy of consideration. Since many of the markers investigated suffer from low specificity, the goal of designing such a test is to raise the specificity of the combined marker test relative to the specificities of the individual markers used. This goal could be best achieved if the levels of the second marker were determined only for the subjects who gave positive results for the first marker measured, and if the two markers chosen were those with the highest possible sensitivity. As an example of such multiple marker tests, the three markers of table III chosen by the authors include those with the highest sensitivities. The overall sen-
sensitivities, specificities, predictive values and efficiencies of the six possible two-marker tests have been calculated.

The results of these calculations are shown in table IV. Overall values for the four parameters are calculated as follows. The sensitivity and specificity of the first marker (from table III) are used to calculate the number of true positives, false negatives, true negatives and false positives, as described in the discussion of table I. The true positives and false positives from this first calculation are then used in a second calculation with the sensitivity and specificity of marker two, by the same methods. The results of the second calculation are then combined with the true and false negatives from the first marker. Finally, the overall sensitivity, specificity, predictive value and efficiency are computed as described previously in their definitions. In each case, the overall sensitivity is less than the sensitivity of the individual markers used. This decline is compensated for by the increases in specificity, predictive value and efficiency. It is also of interest to note that the choice of which marker is used to test the entire subject population and which is used only to test those subjects with elevated levels of the first marker has no effect on the overall values of the four parameters calculated.

References


40. Rabow, L. and Kristensson, K.: Changes in lactate dehydrogenase isoenzyme patterns in


