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Sensitivity and specificity of single and combined tumour markers in the diagnosis of leptomeningeal metastasis from breast cancer

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SUMMARY The clinical efficacy of four laboratory tests in detecting leptomeningeal metastases in 57 patients with breast carcinoma was assessed. The sensitivity and specificity of β-glucuronidase, β_2-microglobulin, carcinoembryonic antigen and lactate dehydrogenase in cerebrospinal fluid were determined. As a single test β-glucuronidase was the most sensitive (93%) and specific (93%) for discriminating between leptomeningeal metastases and other CNS metastases from breast cancer. Lactate dehydrogenase was the next most useful marker. Both β_2-microglobulin and carcinoembryonic antigen had a sensitivity of 60%. More specific results were achieved by combining β-glucuronidase and lactate dehydrogenase. CSF β-glucuronidase may be useful by itself and in combination with lactate dehydrogenase in the detection of leptomeningeal metastases from breast carcinoma.

In recent years the incidence of leptomeningeal metastases from breast carcinoma has steadily increased.[1 2 10 15] This has been attributed to longer survivals achieved with more effective treatment and better neurological diagnostic procedures. Cytological evaluation of the cerebrospinal fluid (CSF) is the principal method of confirming the diagnosis of leptomeningeal metastasis. With the cyto-centrifuge technique, however, cytology will be positive in only 60% of the patients and in 4% there is a false-positive result.[3] In breast cancer the ability to make an early diagnosis of leptomeningeal metastasis was limited by the high proportion of false-negatives in the cytology of CSF.[3–9]

In previous studies we measured the levels of CSF β-glucuronidase, β_2-microglobulin, carcinoembryonic antigen and lactate dehydrogenase in CSF of patients with a variety of metastatic and non metastatic diseases.[11–13] The most important points to consider in the evaluation of a tumour marker are the specificity and sensitivity of the various tests. By specificity is meant the ability of the test to discriminate those individuals who do not have the disease. Sensitivity refers to the ability of the test to detect all patients with the disease.

CSF β-glucuronidase appeared to be an accurate marker for leptomeningeal metastases from solid tumours and β_2-microglobulin the most useful for the detection of leptomeningeal spread from haematological tumours.[11 12] CSF carcinoembryonic antigen assay possesses a low sensitivity but a high specificity in establishing the diagnosis of leptomeningeal metastasis from solid tumours.[13]

We also found that CSF lactate dehydrogenase was a useful marker for leptomeningeal metastasis; however, in contrast to the other tests it was elevated in various non metastatic diseases.[11]

The purpose of the present study was to determine in patients with breast cancer whether a combination of β-glucuronidase, β_2-microglobulin, carcinoembryonic antigen and lactate dehydrogenase measurements is more effective than the use of one of these single tests in the detection of meningeal metastasis.

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Patients and methods

CSF levels of β-glucuronidase, β_2-microglobulin, carcinoembryonic antigen and lactate dehydrogenase were mea-

Table 1 *Positive test results in groups of subjects with and without breast cancer, according to assay for β-glucuronidase, β_2-microglobulin, carcinoembryonic antigen and lactate dehydrogenase*

Group	*No of patients*	*No of positive tests*			
		β-glucuronidase	*β_2-microglobulin*	*Carcinoembryonic antigen*	*Lactate dehydrogenase*
Breast cancer with CNS metastases	41	16	13	12	17
Leptomeningeal	15	13	9	9	14
Spinal	14	1	3	3	1
Parenchymatous	12	2	1	0	2
Breast cancer without CNS metastases	16	0	1	0	0
Neuropathy/plexopathy	8	0	1	0	0
Lumbar disc disease	4	0	0	0	0
Headache	3	0	0	0	0
Cerebro-vascular accident	1	0	0	0	0
Reference range ± 2 SD		9–27 mU/l	0·65–2·2 mg/l	0·8–4 ng/ml	0–26 U/l

sured in 57 women with breast cancer referred to the Antoni van Leeuwenhoekhuis during 1981–1983. Their ages ranged from 30 to 78 years (mean, 57 years). In all patients diagnostic procedures included: CT brain scan, culture and cytological examinations of CSF. They were divided into two groups (table 1). Group I comprised 41 patients with CNS metastases, of whom 15 had leptomeningeal metastases confirmed by positive CSF cytology in 13 and by post-mortem findings in two. Group II comprised 16 patients with neurological disorders, but without demonstrable metastases to the central nervous system (CNS). In order to evaluate this laboratory test it must be possible to discriminate between patients with CNS metastasis (group I) and patients with CNS disease due to other causes (group II).

Samples

CSF samples were centrifuged for cytological examination and immediately analysed for lactate dehydrogenase. The additional CSF samples were coded and stored at −20°C until analysed. Lumbar puncture was performed as a part of clinical diagnostic procedures, and never for the measurement of the markers only.

Assay methods

CSF β-glucuronidase was measured as described earlier,[11] normal values ranged from 9–27 mU/l (table 1). CSF β_2-microglobulin was measured by radio-immuno assay using the Phadebas B_2 Micro Test (Pharmacia Diagnostics, Uppsala, Sweden). β_2-microglobulin increased significantly ($p < 10^{-4}$) with increasing age.[12] No relationship was found with sex. Normal values standardised for age ranged from 0·65 to 2·20 ng/l (table 1). CSF carcinoembryonic antigen was measured by the method of Egan *et al*[19] with several modifications;[13 16] the upper limit of normal is 4 ng per millilitre (table 1).

For β-glucuronidase and carcinoembryonic antigen no relationships were found between test results and age or sex.[11 13] Lactate dehydrogenase determinations were performed using a Dupont ACA, calibrated to give values corresponding with the method of the German Society for Clinical Chemistry.[18] Our test results related to age ($p = 0·036$) and not to sex.[11] The upper limit of normal is less than 26 U/l (table 1).

Statistics

For β_2-microglobulin and lactate dehydrogenase values an unequal deviation was found, so that results were analysed using normal quadratic analysis.[17 18] The sensitivity, specificity and the receiver operator characteristic (ROC) curves were calculated according to Griner *et al.*[14] Galen and Gambino published criteria for evaluating markers in diagnosis and screening.[16] The reference ranges for the four markers in normal CSF were based on mean ± 2 SD (table 1).[11–13]

Results

Table 1 shows the positive results in groups of subjects with and without breast cancer according to assay for β-glucuronidase, β_2-microglobulin, carcinoembryonic antigen and lactate dehydrogenase.

True-positive, that is sensitivity and false-positive rates for the four tumour markers using the upper limit of normal as cut-off point are presented in table 2.

Table 2 *True and false positive rates for four markers of leptomeningeal metastases from breast cancer*

Test	*Cut-off value*	*Sensitivity (true pos. rate)*	*1-specificity (false pos. rate)*
β-glucuronidase mu/l	>27	87	7
β_2-microglobulin mg/l	>2·2	60	12
Carcinoembryonic antigen ng/ml	>4	60	7
Lactate dehydrogenase U/L	>26	93	7

Table 3 *Test characteristics for leptomeningeal metastases from breast cancer*

Test	*Specificity 93%*		*Specificity 100%*	
	Cut-off value	*Sensitivity*	*Cut-off value*	*Sensitivity*
β-glucuronidase mU/l	28	87	39	87
β_2-microglobulin (AS) mg/l	2·9	47	4·4	13
Carcinoembryonic antigen ng/ml	4·05	60	12·05	33
Lactate dehydrogenase U/L	24	93	96	47

Table 4 *Test characteristics for leptomeningeal metastases from solid breast carcinoma*

Test	*Specificity 93%*		*Specificity 100%*	
	Cut-off value	*Sensitivity*	*Cut-off value*	*Sensitivity*
β-glucuronidase mU/l	19	69	17	45
β_2-microglobulin (AS) mg/l	1·6	38	1·00	14
Carcinoembryonic antigen ng/ml	2·05	72	2	26
Lactate dehydrogenase U/L	24	93	23	78

β-glucuronidase, carcinoembryonic antigen and lactate dehydrogenase were found to be relatively specific giving a false positive rate of 7%. Carcinoembryonic antigen and β_2-microglobulin had a similar sensitivity but carcinoembryonic antigen was more specific than β_2-microglobulin; the false-positive rates were 7 and 12%, respectively. Both lactate dehydrogenase and β-glucuronidase were more sensitive markers. Lactate dehydrogenase gave a true-positive rate of 93% and β-glucuronidase of 87% taking the mean plus standard deviation as the upper limit of normal as cut-off point.

Lactate dehydrogenase showed a higher true-positive rate than the other tests, but it is quite difficult to compare these tests directly. We are only able to do this under comparable conditions of the four tests. Therefore we changed the decision level for each test to give specificities (true-negative rates) of 93% and 100% respectively (table 3). We then compared the tests on the basis of the resulting true-positive rates; lactate dehydrogenase was more sensitive than the other tumour markers at a specificity rate of 93%. At a specificity of 100%, however, β-glucuronidase is considerably more sensitive than lactate dehydrogenase. If we chose decision levels needed to give sensitivity rates of 93% or 100% respectively (table 4), then we found that lactate dehydrogenase was more specific than each of the other CSF markers.

Table 5 *Operating characteristics of combination testing*

Test	*Sensitivity*	*Specificity*
		%
β-glucuronidase (A)	87	93
Lactate dehydrogenase (B)	93	93
A or B positive	99	86
A and B positive	81	99

Table 5 shows data concerning the operating characteristics of β-glucuronidase and lactate dehydrogenase when used alone or in combination. The combined tests were most helpful when both markers were normal; the combined specificity was then higher, but the sensitivity was lower. In the fig we constructed a graph that correlates all true- and false-positive rates for a series of cut-off points for different tests, that is β-glucuronidase, β_2-microglobulin, carcinoembryonic antigen and lactate dehydrogenase. Such a graph is known as the receiver operating curve

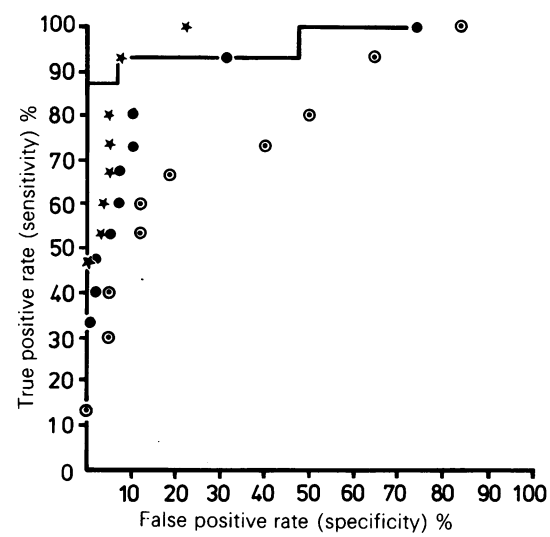

Fig *A receiver operator characteristic curve for β-glucuronidase ———, β_2-microglobulin ⊙⊙⊙⊙, carcino-embryonic antigen ···· and lactate dehydrogenase **** for patients with leptomeningeal metastasis from breast carcinoma.*

(ROC). The curve can be used to decide the optimum cut-off point according to the purpose of the test.

Discussion

A number of CSF markers have been suggested as potential tumour markers for leptomeningeal metastases in solid tumours.[3 4 11-13] For interpretation of this laboratory test sensitivity and specificity calculations are necessary.

Our results regarding sensitivity and specificity for this patient group are summarised in table 2. It shows the limited diagnostic value of CSF carcinoembryonic antigen and β_2-microglobulin with respect to other tests. We standardised the four tests by choosing a decision level so that all tests had the same true-positive rates so that the tests can be directly compared on the basis of their true-negative rates. β-glucuronidase and lactate dehydrogenase showed similar sensitivity rates when we fixed the specificity for the four tests at 93%. However, when choosing decision levels needed to give a 100% specificity, then lactate dehydrogenase was markedly less sensitive (47%) than β-glucuronidase (table 3). A sensitivity of 13% at 100% specificity for β_2-microglobulin makes β_2-microglobulin of little value as a marker for carcinomatosis from breast carcinoma. Its usefulness was not altered by the combination with the other tests. An elevated CSF carcinoembryonic antigen value had a 100% specificity, but the ability to detect meningeal spread from breast carcinoma[1 2 10] is low (sensitivity level 32%) and was unaltered by combination of the test. The ROC curves represented in the figure correlate true- and false-positive rates (sensitivity and 1- minus specificity) for a series of cut-off points for the respective test. Good clinical performance for a test is characterised by a high true-positive rate and a low false-positive rate. β-glucuronidase was the only marker with a high sensitivity at a specificity level of 100%. Lactate dehydrogenase on the other hand, appeared to have in this patient group, a higher sensitivity when the specificity was fixed at 93%, but it had a markedly reduced sensitivity of 47% at 100% specificity. To our knowledge no other study has provided statistical data of the usefulness to combine CSF markers. In patients at risk for leptomeningeal metastases, using the combination of the markers with the highest sensitivity and specificity, that is β-glucuronidase and lactate dehydrogenase respectively, we found an increase in specificity from 93 to 99% at a sensitivity of only 81% (table 5).

In conclusion, when taking into account the factors of sensitivity and specificity in evaluating the four markers, the combined tests of CSF β-glucuronidase and lactate dehydrogenase seem to be the most reliable markers for the detection of leptomeningeal metastases in patients with breast carcinoma.

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