Eur J Cancer Clin Oncol, Vol. 25, No. 12, pp. 1703–1709, 1989.
Printed in Great Britain

0277–5379/89$3.00 + 0.00

Creatine Kinase-BB in the Cerebrospinal Fluid as a Marker of CNS Metastases and Leptomeningeal Carcinomatosis in Patients with Breast Cancer

FLEMMING BACH,* FLEMMING W. BACH,† ANDERS G. PEDERSEN,‡ PIA M. LARSEN* and PER DOMBERNOWSKY*

**Department of Oncology, University Hospital Herlev, †Departments of Clinical Chemistry and Neurology, University Hospital Gentofte and ‡Department of Oncology, Finsen Institute, Copenhagen, Denmark*

Abstract—*To determine whether creatine kinase-BB isoenzyme would be useful in detecting central nervous system metastases secondary to breast cancer, we measured the cerebrospinal fluid (CSF) activity of creatine kinase (CK) and its BB isoenzyme (CK-BB) in 65 consecutive patients suspected of having CNS involvement.*

All patients underwent neurological evaluation, computer tomography (CT) scan and/or radionuclide scintigraphy and lumbar puncture with CSF examination.

Thirty patients had CNS metastases, of whom 18 had parenchymal brain metastases (MET). Twelve had leptomeningeal carcinomatosis (MC), of whom four also had parenchymal brain metastases. Thirty-four patients were concluded not to have CNS involvement, whereas one was considered equivocal.

CK-BB activity was significantly higher in patients with CNS metastases than in those without ($P < 0.05$). This difference was primarily related to the fact that patients with MC had a significantly higher CK-BB activity than patients without CNS metastases or patients with parenchymal brain metastases only ($P < 0.01$ and $P < 0.05$, respectively). Taking 0.20 U/l as a tentative cut-off value (the upper limit range of patients without CNS metastases being 0.19 U/l), 10 out of 12 patients with MC had activities above this level. The sensitivity and specificity for having MC were 83% and 87%, and the positive and negative predictive values 60% and 96%, respectively. The sensitivity and negative predictive value for having any CNS metastases were 57% and 72%. Specificity and positive predictive value: 100%. The CSF activity of CK-BB appears to be a contribution in the diagnosis of MC secondary to breast cancer and seems superior to protein and LDH.

INTRODUCTION

CANCER OF THE BREAST is the most common form of malignant disease in women [1]. Untreated, the mean survival is approximately 2 1/2 years from the diagnosis, and 70–80% of patients will die from their disease [2].

Metastatic spread to the CNS has in recent years been reported with increasing frequency, presumably due to longer survival time and better diagnostic procedures. However, CNS metastases secondary to breast cancer remain undetected until autopsy in nearly 35% of cases [3–7], and in 10% of patients CNS symptoms may be the first manifestation of the malignant disease or recurrence [7, 10]. Leptomeningeal carcinomatosis is diagnosed in 5% of patients with breast cancer *in vivo*, and autopsy studies have yielded a rate of approximately 10% [7–11]. Thus new diagnostic methods are needed to improve our ability to diagnose parenchymal and meningeal CNS metastases.

In the search for better diagnostic methods, various biomarkers have been determined in the CSF of patients with CNS metastases secondary to a variety of malignant diseases [12–17]. CK-BB isoenzyme activity has been reported as significantly elevated in the CSF in patients suffering from leptomeningeal carcinomatosis secondary to small cell lung cancer [18] and in patients with CNS metastases secondary to a variety of other malignancies [19].

Accepted 25 July 1989.
This study was supported by Grant No. 82/1 from The Danish Hospital Foundation for Medical Research, region of Copenhagen, Faeroe Islands and Greenland and The Foundation for Experimental Research in Neurology.
Reprint requests and correspondence to: Flemming Bach M.D., Department of Oncology, University Hospital Herlev, 2730 Herlev, Copenhagen, Denmark.

The aim of the present study is to evaluate whether CK-BB activity in the CSF could be used as a diagnostic marker for parenchymal brain metastases or leptomeningeal carcinomatosis secondary to breast cancer.

MATERIALS AND METHODS

Between June 1985 and January 1988, the activity of CK and its BB isoenzyme were measured in the CSF of consecutive patients suspected of having CNS metastases secondary to breast cancer.

CSF was collected by lumbar puncture (LP) with the patient in the lateral recumbent position. Samples were immediately analyzed for protein, glucose, LDH (enzymatic reaction method, SMAC, upper limit level <80 U/l) and cell counts, and were evaluated cytologically after cytocentrifugation. CSF for CK and CK-BB were frozen and kept at −70°C until analysis. All analyses were made without knowledge of the clinical conclusion.

Cytoplasmic or mitochondrial creatine kinase (CK) are dimers composed of two immunologically distinct sub-units, M and B. They exist in MM, MB and BB forms. The MM and MB isoenzymes are predominant in skeletal and heart muscle, while the BB isoenzyme is localized mainly in brain and smooth muscle. In contrast to the pure muscle type creatine kinase isoenzyme (CK-MM) and the pure brain type isoenzyme (CK-BB), the mixed isoenzyme (CK-MB) is not present in the CSF [20, 21]. Therefore, we considered the activity of CK-B equal to that of CK-BB.

CK and CK-BB activities were determined through a bioluminescence assay using a CK-B kit [(NAC) No. 1243-101, LKB-Wallac, Turku, Finland], with a sample volume of 50 μl. This permitted an increase in sensitivity of 2.5 compared with the standard use of a 20 μl sample volume (because of the linearity of light emission in the bioluminescence assay) [20].

Bioluminescence was measured at 25°C with luminometer 1250 (LKB-Wallac, Turku, Finland). All samples were assayed in duplicate and the results were adjusted for adenylate kinase activity and reagent blank. The coefficient of variation was 6.6% in the total CK assay and 8.4% in the CK-B assay, determined by repeated assay of samples within the normal range of values for total CK and CK-B. The detection limit was 0.04 U/l.

In order to minimize the number of undiagnosed CNS metastases, patients with only sparse neurologic symptoms were also submitted to neurological examination, computer tomography (CT) scan of the brain, and/or radionuclide scintigraphy and lumbar puncture.

Post mortem examination of the brain was carried out whenever possible The cerebrum was cut by frontal sections in 0.5–1 cm thick slices. The cerebellum, brainstem and pituitary gland were cut by horizontal sections in 0.4–0.5 cm thick slices. For histological examination, tissue was also taken from macroscopically suspicious areas. The tissue was stained with hematoxylin–eosin and periodic acid–Schiff stains (PAS).

Based on these investigations and the clinical course, the patients were allocated into groups with or without metastatic spread to the CNS.

Criteria for metastatic spread to the CNS:

1. *Parenchymal brain metastases* (+*MET*, −*MC*). A positive autopsy performed less than 1 month after the assayed lumbar puncture, or definite neurologic signs of CNS lesion combined with a positive scan result, or suggestive neurological signs of a CNS lesion combined with a positive scan and a clinical course compatible with CNS metastases.
2. *Meningeal carcinomatosis* (+*MC*). Positive CSF cytology, or an autopsy with carcinomatous leptomeningitis (localized or diffuse) less than 1 month after the assayed lumbar puncture.

Patients having both parenchymal and meningeal metastases were allocated to the group with meningeal carcinomatosis.

Criteria for the exclusion of CNS metastases.

1. −*CNS metastases*. A negative brain autopsy in patients who had no treatment of possible metastases after the assayed lumbar puncture.
2. Prolonged survival (>6 months) in patients who had no CNS symptoms and no treatment directed against possible CNS metastases.

Patients who did not qualify for either of the two main categories were considered equivocal and were excluded from the statistical evaluation.

The Mann–Whitney test was used for the statistical analysis of differences between the groups. In evaluating the diagnostic value of CK-BB, the terminology described by Griener *et al.* was applied [22].

RESULTS

Sixty-five consecutive patients suspected of having CNS metastases were included. Thirty of these had CNS metastases, 16 in the brain only, while two had spinal cord compression due to metastatic spread (CK-BB 0.59 and 0.08 U/l, respectively) and 12 patients had meningeal carcinomatosis. Four of the patients with MC also had parenchymal brain metastases. It was concluded that 34 patients did not have CNS involvement, and one was considered equivocal.

The distribution of patients and CK-BB values are shown in Table 1 and Fig. 1. The median CSF CK-BB activity in the group of patients without CNS metastases was 0.09 U/l. None of the patients had activities exceeding 0.19 U/l, while the median

Table 1. *CK-BB activity in the CSF according to metastatic spread to the CNS in* 64 *patients with advanced breast cancer*

	No. of patients	Median (U/l)	Range (U/l)	Tentative elevated
Any CNS metastases	30	0.23	0.04–12.30	57%
Parenchymal brain metastases	18	0.14	0.04– 0.83	39%
Leptomeningeal carcinomatosis	12	0.49	0.08–12.30	83%
Absence of CNS metastases	34	0.09	0.04– 0.19	0%

Tentative cut-off value: 0.20 U/l.
*By definition.

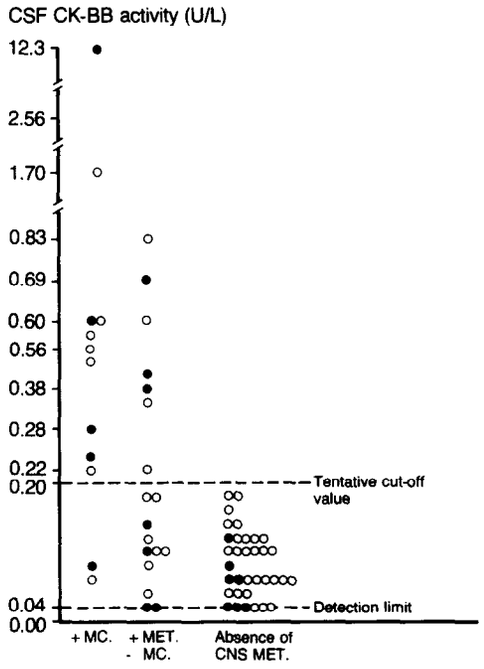

Fig. 1. *Distribution of values of CSF CK-BB activity according to metastatic spread to the CNS. The solid circles represent patients with a CNS autopsy.*

value in patients with MC was 0.49 U/l, with an upper limit of 12.3 U/l. If one uses the upper range limit of patients without CNS metastases as a cut-off point (0.20 U/l), 83% of the patients with MC and 57% of the patients with any metastatic spread to the CNS had CSF CK-BB activities above this level. Comparison of these two groups with the group of patients without CNS metastases shows significant differences ($P < 0.01$, and $P < 0.05$ respectively).

Furthermore, Table 2 shows the distribution of total CSF CK. The median activities of CK are well correlated to that of CK-BB, and the median CK-BB/CK ratio is similar throughout the various groups of patients. With a cut-off value of 0.20 U/l for CK-BB, the sensitivity, specificity, positive and negative predictive values for having any CNS metastases are 57%, 100%, 100% and 72% respectively (Table 3).

When evaluating the possibility of discriminating between patients with MC and MET or no CNS metastases, only 39% of parenchymal metastases (MET) were detected with this cut-off level, and a sensitivity of 83% was found when comparing MC v. MET or no metastases (Tables 3 and 4). Taking these values into account, one has to classify CK-BB levels above 0.20 U/l as false positive for MC and MET respectively, when calculating the specificities and positive predictive values, and CK-BB levels below 0.20 U/l as false negative for MC and MET respectively, when calculating the negative predictive values. As illustrated in Table 4, elevated CSF CK-BB activity is also suggested to be superior to CSF protein, LDH and glucose in diagnosing MC.

Since the cut-off point is arbitrarily set, we examined the relative sensitivity and specificity at different cut-off points and compared it to protein and LDH values. The receiver operating characteristic (ROC) curves of CK-BB, when discriminating between patients with and without MC, with and without MET, and between the presence or absence of any CNS metastases, are illustrated in Fig. 2. These curves describe the correlated true and false positive rates for a series of cut-off points. Good clinical performance for a test is characterized by a simultaneously high true positive rate and low false positive rate, and only in diagnosing MC does CK-BB seem to have a high sensitivity (true positive rate) and low false positive rate. In addition, when two or more tests are available to pursue a particular diagnosis, a comparison of the ROC curve of each will often show where one has the advantage over the other. It can be seen that the ability of CK-BB with any given false positive rate is more sensitive than LDH and protein in diagnosing MC (Fig. 3).

Of the 12 patients with leptomeningeal carcinomatosis all except one patient, who had MC at autopsy less than 1 month after the LP (CK-BB 0.04 U/l), had their MC diagnosed clinically, although four of the patients had two lumbar punctures and the patient with the CSF CK-BB activity at 12.3 U/l had three repeated lumbar punctures before tumor cells were demonstrated.

Table 2. Total CK enzyme activity in the CSF according to metastatic spread to the CNS in 64 patients with advanced breast cancer

	No. of patients	Median (U/l)	Range (U/l)	CK-BB of total CK (% of median)
Any CNS metastases	30	0.34	0.04–12.80	68%
Parenchymal brain metastases	18	0.22	0.04–0.93	64%
Leptomeningeal carinomatosis	12	0.61	0.04–12.80	81%
Absence of CNS metastases	34	0.16	0.04–0.57	63%

Table 3. Sensitivity, specificity, positive and negative predictive values of CK-BB activity in the CSF, in cases of any CNS metastases, parenchymal brain metastases or leptomeningeal carcinomatosis

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Any CNS metastases	57%	100%*	100%*	72%
Parenchymal brain metastases	39%	78%	41%	77%
Leptomeningeal carcinomatosis	83%	87%	60%	96%

Tentative cut-off value 0.20 U/l

Sensitivity: true positive rate	A/(A + C).
Specificity: true negative rate	D/(B + D).
Positive predictive value. Diagnostic specificity:	A/(A + B).
Negative predictive value. Diagnostic sensitivity:	D/(C + D).

		True diagnosis	
		Pos.	Neg.
Test result	Pos.	A	B
	Neg.	C	D

*By definition.

Table 4. Sensitivity, specificity, positive and negative predictive values of CK-BB, LDH, protein and glucose in the CSF in breast cancer patients having any CNS metastases, parenchymal brain metastases or leptomeningeal carcinomatosis

Cut-off value	CK-BB (U/l) >0.2	LDH (U/l) >80	Protein (g/l) >0.8	Glucose (mmol/l) <2.0
Any CNS metastases				
Sensitivity	57%	47%	40%	6%
Specificity	100%	92%	94%	100%
Positive predictive value	100%	88%	89%	100%
Negative predictive value	72%	60%	57%	53%
Parenchymal brain metastases				
Sensitivity	39%	33%	34%	0%
Specificity	78%	79%	79%	97%
Positive predictive value	41%	38%	44%	0%
Negative predictive value	77%	71%	76%	76%
Leptomeningeal carinomatosis				
Sensitivity	83%	50%	67%	17%
Specificity	87%	77%	82%	100%
Positive predictive value	60%	38%	44%	100%
Negative predictive value	96%	85%	92%	82%

A total of 19 patients (30%), marked with solid circles in Fig. 1, had CNS autopsies. One patient classified in the group 'without' CNS metastases had MC at autopsy 26 months after the LP. None of the remaining autopsies were in contrast to the clinical conclusions.

It was concluded that the equivocal patient had a cerebral hemorrhage based on the clinical features and radionuclide scintigrams. In this patient, autopsy was not performed (CK-BB 2.52 U/l).

DISCUSSION

The present study demonstrates a significant increase in the activity of CK-BB in CSF from patients with MET and MC secondary to breast cancer.

Rubery *et al.* [23] found that the amount of tumor burden in breast cancer patients correlated well with the level of serum CK-BB. In fact, an elevated plasma CK-BB level was recorded in 63% of patients with extensive tumor burdens. In their study, the origin of the CK-BB was not quite clear since the brain could not be excluded as the source of the raised serum CK-BB, and it was not known whether any of these patients had CNS metastases. In the

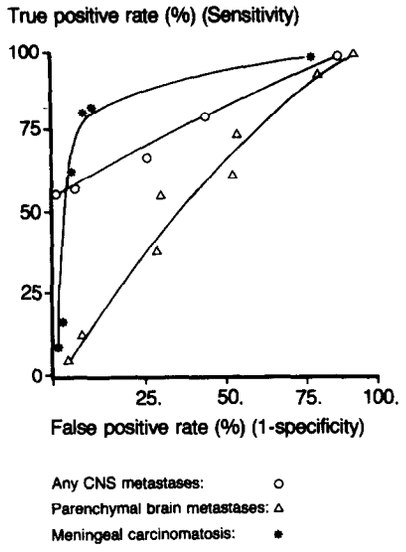

Fig. 2. Receiver operating characteristic (ROC) curves of CK-BB isoenzyme in the CSF in cases of meningeal carinomatosis or parenchymal brain metastases, and discriminating between the presence and absence of any CNS metastases.

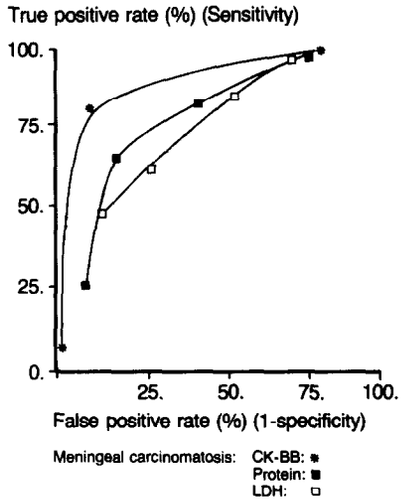

Fig. 3. Receiver operating characteristic (ROC) curves of CK-BB isoenzyme, LDH and protein in the CSF in patients with leptomeningeal carinomatosis.

present study, a similar frequency (57%) of elevated CK-BB in the CSF has been found in patients with CNS metastases in general. None of the patients without metastatic spread to the CNS had CSF CK-BB activity exceeding 0.19 U/l. Eighty-three per cent of the patients with MC and 39% of the patients with parenchymal brain metastases were above the tentative cut-off level.

These observations in patients with breast cancer are in accordance with a study of patients with MET and MC from small cell lung cancer suffering from MET and MC, where increased activity of CK-BB in the CSF also was found [18].

The origin of the increased CK-BB activity in CSF could be damaged brain tissue or tumor cells. Brain tissue damage can cause elevation of CSF CK-BB activity [24] however; especially SCLC cells contain substantial amounts of CK-BB [25]. The contribution of the CK-BB activity in small cell lung cancer consequently could be a combination of CK-BB emanating from tumor cells and damaged brain tissue. No such data are available on breast cancer cells, but the similarity between the results obtained in the study of small cell lung cancer [18] and in the present study may favor damaged brain tissue as the source of CSF CK-BB. This view is in agreement with a recent study where elevated levels of CK-BB and myelin basic protein were found in the CSF secondary to CNS metastases, including both MET and MC from a variety of solid tumors [19].

Other kinds of brain damage, e.g. cerebrovascular lesions, may increase the CK-BB activity in the CSF [26]. The clinical findings including CT scans in the present study makes this possibility unlikely except in the equivocal patient. Additionally, in our previous study of 75 SCLC patients, only one patient had cerebrovascular disorders.

Concerning the false negative patients with parenchymal brain metastases, the anatomical localization of the metastases and brain tissue damage without the possibility of leakage of CK-BB into the CSF could be an explanation.

No plasma activity was determined in the present study, but the fact is that the CK-BB/CK ratio is somewhat higher in MC patients than in patients without CNS metastases (Table 2). This supports the theory of a local origin rather than a spillover, since the CK-MM fraction would otherwise be much higher (normal CK-MM activity in the blood is 50–100 times higher than in the CSF). Supporting this, the MC patient with CK-BB activity at 12.3 U/l was without extracerebral malignant disease and parenchymal brain metastases.

Diagnosing MC is difficult, and no single method can exclude the diagnosis. The finding of tumor cells in the CSF is generally considered diagnostic for MC, and in inter-observer studies, pathologists seem to be reliable observers [27]. In most studies, some degree of meningeal involvement was always found during autopsy in patients with tumor cells in the CSF [28, 29]. Wasserstrom *et al.* documented malignant cells in the CSF of only 54% out of 90 patients with MC at autopsy secondary to a variety of solid tumors using a single LP. An additional 30% were diagnosed by a second LP [8]. However, this statement is critically dependent on the indication for carrying out lumbar punctures. In the present study, LP was instigated at rather liberal indications in order to pick up every case of MC. Where there was any doubt the lumbar punctures were repeated to increase the likelihood of detecting MC. We feel certain that in accordance with the

study criteria, we have not overlooked patients with MC in the group 'without CNS metastases'. In contrast, we cannot be sure about the group with parenchymal brain metastases since the patients received cranial irradiation after the diagnosis was established and since only one-third of these patients had a CNS autopsy performed. All but one of the MC patients had the diagnosis established clinically; however, four of the patients needed two lumbar punctures, and the patient with the CK-BB at 12.3 U/l was first diagnosed by the third repeated lumbar puncture.

Because of the small number of CNS autopsies, this study cannot be used for comparison of CSF cytology and CK-BB as a diagnostic tool. However, we consider that a marker of MC as mentioned is a valuable supplement to CSF cytology, which can only be expected to have a sensitivity at 60% by the first lumbar puncture [8].

Apart from routinely used markers such as protein, glucose and LDH, Twijnstra *et al.* found a sensitivity and specificity at 87% and 93% for beta-glucoronidase in the CSF in patients with MC secondary to breast cancer and concluded that the combination of CSF beta-glucoronidase and LDH was the most reliable marker for detecting MC [17]. In the present study, the ability to diagnose MC using CK-BB is similar, although an elevated activity of CK-BB in the CSF does not distinguish between MC and parenchymal brain metastases and thus, by itself, cannot guide treatment. Ongoing studies have demonstrated an explicit correlation between response to treatment and decrease of CSF tumor markers, including CK-BB, in MC patients treated with intrathecal Methotrexate [30].

In conclusion, low activity of CK-BB in the CSF in patients with breast cancer seems to exclude MC. Increased CK-BB activity, particularly in patients without signs of cerebrovascular lesions or parenchymal brain metastases, is highly indicative of MC, although additional information on this marker is needed before its definite diagnostic role can be established.

REFERENCES

1. Duncan W. *Treatment of Cancer.* London, Chapman and Hall, 1984, 329.
2. Haagensen CD. *Diseases of the Breast.* Philadelphia, WB Saunders, 1971, 458.
3. Chason JL, Walker FB, Landers JW. Metastatic carcinoma of the central nervous system and the dorsal root ganglia. *Cancer* 1963, **16**, 781.
4. Less S, Netsky MG. Metastases of neoplasms to the central nervous system and meninges. *AMA Arch Neurol Psychiat* 1954, **72**, 133–153.
5. Vieth RG, Odom GL. Intracranial metastases and their neurosurgical treatment. *J Neurosurg* 1965, **23**, 375–387.
6. Posner J. Brain metastases. A clinician's view. In: Weiss, Gilbert, Posner, eds. *Brain Metastases.* The Hague, Martinus Nijhoff.
7. Yap HY, Yap BS, Tashima CP *et al.* Meningeal carcinomatosis in breast cancer. *Cancer* 1978, **42**, 283–286.
8. Wasserstrom WR, Glass JP, Posner JB. Diagnosis and treatment of leptomeningeal carcinomatosis from solid tumours. Experience with 90 patients. *Cancer* 1982, **49**, 759–772.
9. Yap HY, Yap B, Rasmussen S *et al.* Treatment for meningeal carcinomatosis in breast cancer. *Cancer* 1982, **49**, 219–222.
10. Ongerboer de Visser BW, Somers R, Nooyen WH *et al. Neurology* 1983, **33**, 1565–1572.
11. Barron K, Hirano A, Shukuro A *et al.* Experiences with metastatic neoplasms involving the spinal cord. *Neurology* 1956, **9**, 91–106.
12. Schold SC, Wasserstrom WR, Fleisher M *et al.* Cerebro fluid biochemical markers of central nervous metastases. *Ann Neurol* 1980, **8**, 597–604.
13. Ongerboer de Visser BW, Zanten AP van, Twijnstra A *et al.* Sensitivity and specificity of cerebrospinal fluid markers of central nervous system metastases. *Prog Exp Tumor Res* 1985, **29**, 105–115.
14. Zanten AP van, Twijnstra A, Ongerboer de Visser BW *et al.* Tumour markers in the cerebrospinal fluid of patients with central nervous system metastases from extracranial malignancies. *Clin Chim Acta* 1988, **175**, 157–166.
15. Pedersen AG, Becker KL, Bach F *et al.* Cerebrospinal fluid bombesin and calcitonin in patients with central nervous system metastases from small cell lung cancer. *J Clinical Oncol* 1986, 1620–1627.
16. Pedersen AG, Marangos P, Gazdar P *et al.* Neuron specific enolase in the cerebrospinal fluid as a marker of CNS metastases in small cell lung cancer. *Proc ASCO* 1984, C-**25**.
17. Twijnstra A, Zanten-Van AP, Nooyen WJ. Sensitivity and specificity of single and combined tumour markers in the diagnosis of leptomeningeal metastases from breast cancer. *J Neurol Neurosurg Psychiat* 1986, **49**, 1246–1250.
18. Pedersen AG, Bach F, Nissen M *et al.* Creatine kinase-BB and beta-2-microglobulin as markers of CNS metastases in small cell lung cancer. *J Clin Oncol* 1985, **10**, 1364–1372.
19. Mathias-Guiu J, Martinez-Vazques J, Ruibal A *et al.* Myelin basic protein and creatine kinase-BB isoenzyme as CSF markers of intracranial tumors and stroke. *Acta Neurol Scand* 1986, **73**, 461–465.

22. Halonen T, Nanto V, Frey H *et al.* Bioluminescent assay of total and brain specific creatine kinase activity in cerebrospinal fluid. *J Neurochem* 1982, **39**, 36–43.
21. Lundar T, Stokke O. Total creatine kinase activity in cerebrospinal fluid as an indicator of brain damage during open heart surgery. *Scand J Thorac Cardiovasc Surg* 1983, **17**, 157–161.
22. Griner PF, Mayewski RJ, Mushlin AI. Selection and interpretation of diagnostic tests and procedures. *Ann Intern Med* 1981, **94**, 553–600.
23. Rubery ED, Doran JF, Thompson RJ. Creatine kinase-BB isoenzyme as a tumor marker in breast cancer, lung cancer and lymphoma. *Ann NY Acad Sci* 1983, **417**, 410–426.
24. Bach FW, Kruse A, Melgaard B *et al.* Creatine kinase-BB release into cerebrospinal fluid after lateral ventricle cannulation. *Br J Neurosurg* 1988, **2**, 339–342.
25. Gazdar A, Zweig MH, Carney D *et al.* Levels of creatine kinase and its BB isoenzyme in lung cancer specimens and cultures. *Cancer Res* 1981, **41**, 2773–2777.
26. Wevers RA, Jansen PHP, Van Woerkom LMJ *et al.* The significance of total creatine kinase activity and isoenzyme determinations in cerebrospinal fluid of neurological patients. *Clin Chim Acta* 1984, **143**, 193–201.
27. Pedersen AG, Olsen J, Nasiell M. The cytologic diagnosis of meningeal carcinomatosis from small cell lung cancer. A variability study of CSF cytology. (Submitted for publication.)
28. Choi HH, Anderson PJ. Diagnostic cytology of cerebrospinal fluid by the cytocentrifuge method. *Am J Clin Pathol* 1979, **72**, 931–943.
29. Glass JP, Melamed N, Chernick NL *et al.* Malignant cells in the cerebrospinal fluid (CSF). The meaning of a positive CSF cytology. *Neurology* 1979, **29**, 1369–1375.
30. Bach F, Soletormos G, Bach FW *et al.* TPA and CK-BB. New tumour markers of leptomeningeal carcinomatosis secondary to breast cancer. (Submitted for publication.)