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Brain-type Creatine Kinase BB as a Potential Tumour Marker—Serum Levels Measured by Radioimmunoassay in 1015 Patients with Histologically Confirmed Malignancies*

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Abstract—*Serum CK-BB levels have been assayed in 1015 patients with histologically confirmed malignancy. Thirty-four per cent of these patients had elevated serum CK-BB levels when compared with the levels found in a population of 1006 blood donors. In breast cancer patients tumour burden correlated well with the degree of elevation of the serum CK-BB, with a mean serum level of 2.01 μg/l (range 0.0–7.7 μg/l) in patients post-mastectomy, rising to a mean of 4.78 μg/l (range 0.1–37 μg/l) in patients with metastatic disease. In carcinoma of the bronchus elevated levels occurred in 41% of patients, no correlation with apparent stage of disease being detected. In lymphomas moderate elevations of CK-BB occurred in 34% of patients and serial measurements correlated with response to therapy. Elevated levels occurred with normal ESR levels. In cancer of the bladder, prostate, testis and head and neck, raised CK-BB levels occurred more frequently in patients with metastatic disease than in those thought to have local disease alone, while in sarcoma, cancer of the ovary, uterus, cervix, stomach, bowel and anal canal the presence of persistent disease correlated with a raised CK-BB level. Radiotherapy to normal tissues containing CK-BB did not elevate serum CK-BB levels but irradiation of tumour tissue containing CK-BB (e.g. prostatic carcinoma or lymphoma) resulted in transient elevations of serum CK-BB. These results suggest CK-BB warrants further investigation as a monitor of response to therapy and as a marker of persistent or metastatic disease in a variety of tumours.*

INTRODUCTION

SINCE the original report by Coolen [1] of elevated brain-type creatine kinase isoenzyme (CK-BB) in the serum of two patients with oat-cell carcinoma of the lung, several investigators have suggested that measurement of this isoenzyme may be of value as a diagnostic tumour marker in a variety of malignant conditions [2]. The development of sensitive radioimmunoassays for CK-BB [3, 4] has meant that the incidence of raised circulating levels of this protein in malignant disease can now be more critically examined. Raised levels of immunoreactive CK-BB have been found in a high proportion of breast and prostate carcinomas, especially in the presence of overt metastases [5, 6]. We have previously reported a study of serum CK-BB levels measured by radioimmunoassay in women with breast cancer and have proposed that this isoenzyme may be of special value in detecting residual disease [7]. We now report the levels of immunoreactive serum CK-BB in 1015 patients with histologically confirmed malignant disease.

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MATERIALS AND METHODS

Patients

All patients were attending the Radiotherapeutic Centre, Addenbrooke's Hospital, Cambridge with histologically confirmed malignant disease. All serum samples were separated and stored at −70°C within 4–5 hr of collection and assayed within two weeks. The total patient population was 1015, and a total of 1579 individual CK-BB radioimmunoassay measurements were made on this population. Normal control levels were established by measurement of 1006 blood donor sera as described previously [8].

Radioimmunoassay of CK-BB

The development of a radioimmunoassay for CK-BB has been fully described elsewhere [4]. Intra- and inter-assay variations were 4.5 and 10.5% respectively. Preliminary experiments (Rubery *et al.*, in preparation) characterizing serum CK-BB immunoreactivity according to criteria discussed elsewhere [9] have shown that the measured immunoreactivity has the same molecular weight and charge characteristics as native CK-BB; in particular, we can find no evidence that raised levels are due to the mitochondrial form of the enzyme recently shown to be present in a variety of human tumours [10].

RESULTS

Table 1 summarizes the numbers, diagnostic groups and proportion of patients with raised serum CK-BB levels in the 1015 patients studied. As reported elsewhere [8], we have taken 3 μg/l as the upper limit of normal for serum CK-BB, this is based on the levels found in 1006 blood donor sera, only four (0.003%) of which showed levels above this value [8]. The levels summarized in Table 1 show that raised serum CK-BB levels are found in a wide variety of primary malignancies, with particularly high levels being found in isolated cases of bladder, prostatic, uterine and breast cancer.

Table 1. *Serum CKBB levels in* 1015 *patients with histologically confirmed malignant disease*

Site of primary	Total No. of patients	Percentage with CK-BB > 2.9 μg/l	CK-BB = 0.0–2.9 μg/l No. patients	CK-BB = 0.0–2.9 μg/l Mean ± S.D. (range)	CK-BB = > 2.9 μg/l No. patients	CK-BB = > 2.9 μg/l Mean ± S.D. (range)
Breast	406	34	267	1.17 ± 0.66(0.0–2.9)	139	6.03 ± 5.08(3.0–50.0)
Lung	95	41	56	1.32 ± 0.65(0.3–2.8)	39	6.56 ± 4.23(3.0–19.0)
Hodgkin's disease	34	44	19	0.92 ± 0.54(0.1–2.4)	15	5.69 ± 2.10(3.1–7.8)
Non-Hodgkin's lymphoma	59	29	42	0.99 ± 0.79(0.0–2.9)	17	5.66 ± 2.25(3.0–9.0)
Myeloma	13	23	10	1.09 ± 0.44(0.3–2.1)	3	6.03 ± 2.86(3.3–9.0)
Polycythaemia r.v.	4	0	4	1.42 ± 0.59(0.6–2.5)	0	—
Sarcoma	16	38	10	1.50 ± 0.67(0.7–2.8)	6	4.92 ± 1.26(4.0–7.4)
Bladder	48	25	36	1.01 ± 0.58(0.2–2.5)	12	42.87 ± 103.9(3.2–300*)
Prostate	52	44	29	1.34 ± 0.68(0.3–2.3)	23	28.00 ± 50.46(3.3–190.0)
Testes	36	33	24	0.93 ± 0.46(0.4–2.2)	12	5.96 ± 4.98(3.0–20.7)
Ovary	41	41	24	1.12 ± 0.90(0.1–2.9)	17	5.04 ± 1.34(3.2–6.8)
Uterus and cervix	50	22	39	0.93 ± 0.56(0.1–2.4)	11	21.50 ± 52.67(3.2–180*)
Head and neck	53	25	40	1.06 ± 0.76(0.1–2.8)	13	4.70 ± 1.86(3.2–9.6)
Brain	12	33	8	1.46 ± 0.51(0.76–2.1)	4	3.98 ± 0.74(3.1–4.8)
Oesophagus	8	63	3	0.70 ± 0.40(0.3–1.1)	5	4.06 ± 0.56(3.4–4.5)
Stomach	8	38	5	1.84 ± 0.98(0.5–2.9)	3	6.80 ± 3.21(3.1–8.8)
Bowel and anal canal	34	32	23	1.08 ± 0.66(0.0–2.8)	11	4.67 ± 1.41(3.2–7.0)
Endocrine	12	17	10	1.07 ± 0.51(0.3–2.4)	2	6.50 ± 0.42(6.2–6.8)
Melanoma	6	33	4	1.15 ± 0.39(0.8–1.7)	2	6.60 ± 2.70(5.0–8.2)
Unknown primary	14	43	8	1.43 ± 0.61(0.6–2.6)	6	6.45 ± 2.65(3.1–10.1)
Kidney	7	29	5	1.36 ± 0.69(0.6–2.4)	2	10.30 ± 5.94(6.1–14.5)
Miscellaneous	7	29	5	0.84 ± 0.86(0.1–2.3)	2	3.95 ± 1.20(3.1–4.8)
Total	1015	34	671		344	

*Elevated mean due to single very elevated result from one patient.

Breast cancer

Thirty-four per cent of the 406 patients in this group had one or more elevated serum CK-BB levels at the time of sampling. Patients with breast cancer were staged according to the 1974 U.I.C.C. TNM classification and placed into four groups (group 1–stage I, II, III patients with a primary lesion still present; group 2—patients originally stage I, II and III who had been treated and were now in complete clinical remission; group 3—patients who had developed local recurrence; group 4—any patient with evidence of metastases) exactly as described previously [7]. Table 2 summarizes serum CK-BB levels in each of these groups and clearly indicates that increased tumour burden is associated with an increased incidence of raised serum CK-BB levels. Of particular importance are group 2 patients, who are post-mastectomy or lumpectomy with no clinical, radiological or scintigraphic evidence of residual disease but who still show raised serum CK-BB levels in 21% of cases. One patient in this group, apparently in complete clinical remission but with a serum CK-BB level of 7.4 μg/l, died one month after sampling from undiagnosed cerebral metastases.

Bronchial carcinoma

Information to enable accurate staging of this group of patients is often incomplete due to their poor general condition. In addition, since it is local policy not to refer patients for radiotherapy after complete surgical resection of non-oat-cell carcinoma, and to irradiate all patients with oat-cell carcinoma without surgery, it is difficult to define a 'disease-free' population. Because of these limitations patients were divided into those thought to have local disease and those thought to have metastases at the time of serum sampling, and the results are summarized in Table 3. There was little difference in serum levels between the two groups, except for a smaller percentage of patients with raised levels in the 'local disease' group (Table 3). Further analysis of these patients to correlate CK-BB levels with tumour histology, prognosis and sex of the patient is in progress.

Lymphomas

In this series CK-BB elevation occurred in 54% of patients with lymphoma, with significantly more elevated levels in patients with Hodgkin's disease than in the non-Hodgkin's lymphomas (Table 1). In several patients there was evidence that the CK-BB level acted as a monitor of disease status, e.g. a 30-year-old man with IIIb Hodgkin's disease appeared in complete remission (CR), with normal ESR, CXR and lymphangiogram after quadruple chemotherapy and radiotherapy to site of original disease, but with an elevated CK-BB level of 5.6 μg/l. Three months later he relapsed with glands in the neck. Following further radiotherapy to this area he returned to CR status and his serum CK-BB fell to 0.7 μg/l. He has subsequently remained in CR with normal serum CK-BB for two years. Another patient with massive abdominal disease from a non-Hodgkin's lymphoma and with a CK-BB level of 3.7 μg/l had a rise of serum levels to 7.4 μg/l during radiotherapy to the whole abdomen (Fig. 1), followed by a decline to normal levels after radiotherapy. In contrast, the few patients available with myeloma and polycythaemia rubra vera had a lower incidence of raised serum CK-BB levels (Table 1).

Sarcomas

This was inevitably a small group of patients due to the rarity of the disease. However, when the results were grouped according to whether disease was clinically present at the time of

Table 2. CK-BB levels in carcinoma of breast according to stage of disease

Group	Disease status	CK-BB < 3.0 μg/l	CK-BB > 3.0 μg/l	Total No. patients	Per cent with elevated CK-BB	Mean μg/l ± S.D. (range)
I	Stage I/II/III: primary *in situ*	21	7	28	25	2.43 ± 1.87(0.3–7.9)
II	Stage I/II/III: post-mastectomy	195	53	248	21	2.01 ± 1.57(0.0–7.7)
III	Local recurrence	32	28	60	47	3.70 ± 6.40(0.1–50)
IV	Distant metastases	37	63	100	63	4.78 ± 4.50(0.1–37)

No patient contributed more than one sample to any of the groups. Where more than one result was available the most recent sample taken was used.

Table 3. CK-BB levels in carcinoma of the bronchus according to extent of disease

Stage	CK-BB > 3.0 μg/l	Total No. patients	Per cent CK-BB > 3.0 μg/l	Mean (μg/l) ± S.D. (range)
Stage I/II/III	24	70	34	6.9 ± 4.4(3.0–19.0)
Stage IV	13	28	46	6.8 ± 4.4(3.3–17.0)

No patient contributed more than one sample to either group. Where more than one result was available the most recent sample taken was used.

assay, 2 out of 5 patients (40%) with disease present had elevated levels while only 2 out of 12 patients (17%) free of disease had elevated levels. One of these had an elevated level of 7.4 μg/l on day 20 of radiotherapy to the arm following incomplete surgical removal of a synovial sarcoma. This level fell to 0.86 μg/l in the post-radiotherapy period, suggesting that the radiotherapy had caused lysis of residual disease. The remaining patient had a persistent raised alkaline phosphatase as well as an elevated CK-BB level of 4.9 μg/l, in spite of being in CR for 3 years following surgery and radiotherapy for a malignant fibrous histiocytoma in the abdomen.

Carcinoma of the bladder and prostate

The incidence of raised levels among cases of carcinoma of the bladder was rather lower than most groups (25%). Possibly this reflects the tendency of this cancer to be a locally indolent disease for some years. Only 4 out of the 48 patients in this group had metastatic disease, and of these three had elevated CK-BB levels of 7.0, 9.8 and 4.0 μg/l. One patient had an extremely high serum CK-BB level of 300 μg/l. He had poorly differentiated transitional cell carcinoma of the bladder, but was also noted to have gross hepatosplenomegaly of unknown etiology. Of the patients with carcinoma of the prostate, those with evidence of metastatic disease (32 patients) showed a 55% incidence of raised levels (5.4–190 μg/l), but only 1 out of 20 patients thought to have local disease showed a raised level (5.4 μg/l).

Testicular cancer

One hundred and thirteen samples from 36 patients were assayed. The large number of samples per patient reflects the close monitoring of serum AFP and HCG in these patients during radiotherapy, chemotherapy and follow-up.

In the present sample, 17 out of 18 patients in complete remission and on no therapy had normal serum CK-BB levels, with the remaining patient showing a marginal level of 3.1 μg/l. Effective therapy in this disease results in a high complete remission rate [11] and these patients are believed to be cured of their disease. Two out of 4 patients receiving chemotherapy for disseminated disease had elevated CK-BB levels of 4.4 and 4.0 μg/l, both of which fell to normal following chemotherapy resulting in CR status. Six out of 23 patients having para-aortic node radiotherapy for stage I and II disease had elevated levels which fell to normal during radiotherapy in 4 cases. A fifth patient, whose CK-BB level was 3.3 μg/l on day 8 of radiotherapy, had no subsequent samples taken. The final patient started with a serum CK-BB level of 12 μg/l prior to radiotherapy for stage I embryonal carcinoma. Serum CK-BB levels fluctuated widely during radiotherapy and have continued to do so on follow-up, an isolated level of 20.7 μg/l being detected at 9 months post-radiotherapy. Serum CEA levels have also fluctuated widely over the same period, but AFP and βHCG levels have been normal at all times, as has his liver scan. The patient at the moment is in complete remission two years after therapy.

Carcinoma of the ovary, uterus and cervix

When the total number of patients (41) with carcinoma of the ovary were regrouped according to disease status, only 1 out of 26 patients in complete remission showed a raised level of CK-BB at 4.8 μg/l, while 11 out of 20 patients (55%) with persistent disease showed raised levels. The low incidence of raised levels seen in carcinoma of the uterus and cervix (Table 1) possibly reflects early presentation and frequently effective treatment. Three out of 18 patients considered free of disease had raised levels of 3.3, 3.8 and 4.8 μg/l, while of 15

patients with advanced disease, 4 showed raised levels of 7.4, 8.2, 14.0 and 180 μg/l.

Head and neck

This is another site where disease tends to be responsive to therapy and where metastases appear late. Only 2 patients had metastatic disease, both to bones and both having elevated levels of serum CK-BB (4.5 and 9.4 μg/l). None of the 7 patients in complete remission had raised levels, while 9 out of 45 patients (20%) with local disease had elevations ranging from 3.2 to 6.4 μg/l.

Cerebral tumours

Although cerebral trauma has been shown to produce raised serum CK-BB [8], only 4 out of 12 patients with primary cerebral tumours had raised levels, with the highest at 4.8 μg/l. Six out of 9 patients with cerebral metastases had raised levels (3.3–10.1 μg/l), this presumably reflecting the generalized tumour burden rather than the specific localization of metastases in the cerebrum.

Carcinoma of the oesophagus, stomach, bowel and anal canal

All the 8 patients with carcinoma of the oesophagus had extensive disease but the highest level shown was 4.8 μg/l, despite 5 of the patients being assayed within a month of death. Two patients with adenocarcinoma of the stomach showed levels of 8.8 and 8.5 μg/l following incomplete surgical removal, one of these patients showing a fall to 0.9 μg/l following radiotherapy but with a subsequent rise to 4.5 μg/l one month before death. Nine out of 27 patients (33%) with persistent carcinoma of the bowel or anal canal had levels above 3.0 μg/l, while 2 out of 9 patients (22%) in clinical remission had elevated levels. One patient had normal levels for 21 months following surgery and 5-fluorouracil for small bowel cancer, but elevated levels in the absence of any symptoms or signs at 24 and 26 months after treatment.

Effect of radiotherapy on serum CK-BB levels

Many of the serum samples were taken while patients were receiving radiotherapy, and therefore the effect of irradiation on serum CK-BB levels must be considered. Radiotherapy to CK-BB-containing tissues could raise serum CK-BB by leakage from normal cells, while radiotherapy to tumour tissue could raise serum CK-BB by lysis of malignant cells. Brain, bladder and intestine are normal tissues containing relatively high amounts of CK-BB (> 100 U/g) [2]. Nine patients with cerebral or bladder tumours with initially normal serum CK-BB levels were serially sampled during radiotherapy to the primary site. In each case the serum CK-BB level failed to show any significant rise during radiotherapy. However, several patients with initially elevated serum CK-BB showed higher levels during radiotherapy (see Fig. 1).

DISCUSSION

The present study clearly shows that elevated levels of serum CK-BB can be found in a variety of malignant diseases. It is assumed that the source of the raised serum CK-BB is the tumour tissue itself; radiotherapy *per se* does not seem to produce raised levels, and within the large group of women with breast cancer the level of serum CK-BB correlates closely with the tumour burden (Table 2).

Two previous studies on considerably smaller patient populations have shown the presence of CK-BB in malignant sera both by radioimmunoassay [12] and by electrophoresis [2], with raised levels being especially marked in the presence of metastases [12]. Since brain-type CK-BB appears to be produced by a wide variety of tumours (possibly due to its foetal nature) [2], the potential value of serum CK-BB as a screening test for a particular cancer is clearly limited. Furthermore, raised serum levels of this protein are known to occur in other clinical situations, e.g. neurological disorder [4] and head injuries [8]. We would also emphasize that the control population in the present study were blood donors (in whom raised serum CK-BB levels are rare [8]) and the incidence of raised serum CK-BB in a popu-

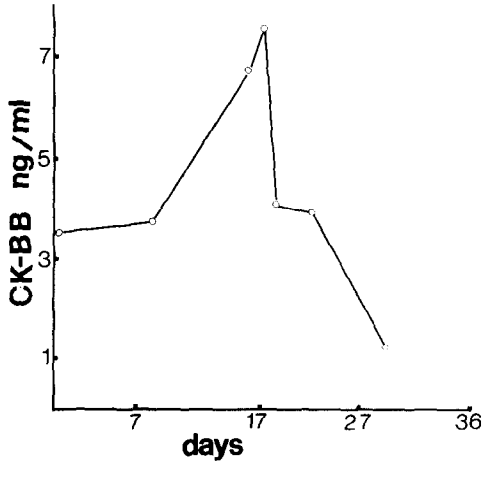

Fig. 1. Serial serum CK-BB levels in a patient (J.S.) with abdominal lymphoma undergoing whole abdomen radiotherapy.

lation with proven benign disease needs further study. Previous reports suggest that this incidence is low [5, 12]; however, we are currently analysing serum CK-BB levels in 1000 patients attending out-patient clinics with clinically benign conditions. Despite these reservations, the present study has shown that one in three patients with histologically confirmed malignant disease has a raised level of serum CK-BB. The potential of this new tumour marker as a monitor of treatment and as an early predictive index of recurrence deserves further investigation.

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