Elevated Serum Creatine Kinase BB Levels in Patients with Small Cell Lung Cancer

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ABSTRACT

Clinical tumor specimens and cultures of small cell lung cancer (SCLC) produce 10- to 100-fold higher quantities of the BB isoenzyme of creatine kinase (CK-BB) (EC 2.7.3.2) than did other types of lung cancer. Serum CK-BB levels were evaluated in 105 newly diagnosed, previously untreated patients with SCLC. All patients were thoroughly staged, including 42 patients with limited-stage and 63 patients with extensive-stage disease. Serum CK-BB was elevated (>10 ng/ml) in 27 patients (26%) (range, 11 to 522 ng/ml; median, 40 ng/ml). Only 1 of 42 patients with limited disease had an elevated serum CK-BB, while 26 of 63 (41%) of patients with extensive disease did. When patients were subgrouped according to the number of metastatic sites detected in pretreatment staging, a significant association between the presence of an elevated serum CK-BB and the number of metastatic sites was observed (p < 0.005). No association between the presence of metastatic disease in a specific site and an elevated serum CK-BB could be detected. After adjusting for the number of metastatic sites, survival among patients with a normal pretreatment CK-BB was significantly better than in patients with an elevated CK-BB (p = 0.014). Sequential serum CK-BB determinations in 33 patients revealed an excellent correlation between clinical response to therapy and serum CK-BB levels. Continuous SCLC cell lines established from 13 patients in this study all expressed high levels of CK-BB. These data suggest that serum CK-BB determinations may be of value in estimating the extent of tumor dissemination, assigning prognosis, and monitoring response to therapy in patients with SCLC.

INTRODUCTION

SCLC accounts for 20 to 25% of all new cases of primary lung cancer in the United States (7). Although the vast majority of patients will achieve a clinical remission with current intensive combination chemotherapy, with or without concomitant radiation therapy, for most patients tumor relapse occurs, and only 5 to 10% of all patients may be cured of their disease. While several serum components have been proposed as "biomarkers" of SCLC or as indicators of the extent of disease at diagnosis and monitors of response to therapy, at the present time none seem either sensitive or specific enough to mandate their widespread use either in the management of patients or in screening for early detection (1, 18, 20-24, 28, 29, 32, 34, 45-50).

In vivo and in vitro studies of SCLC have demonstrated that it possesses a wide range of properties associated with cells of the APUD system, including high levels of the key APUD enzyme L-dopa decarboxylase (4, 5, 8, 16), neurosecretory granules (6, 16, 35), the production of a variety of hormones and polypeptides (41), and the expression of NSE (31). Recently, we have demonstrated in in vitro cultures of SCLC that this tumor expresses large amounts of CK-BB (17). CK-BB levels in both clinical specimens and established cell lines of SCLC were 10- to 100-fold greater than in normal lung tissue and in non-SCLC lung tumors and cell lines, suggesting that CK-BB may be a useful marker for SCLC. The expression of CK-BB in the cell lines of SCLC showed an excellent correlation with the presence of L-dopa decarboxylase activity in the tumor cells.

Creantin kinase (ATP-creatinine N-phosphotransferase, EC 2.7.3.2) occurs in large amounts in the serum primarily as 3 isoenzymes: CK-BB, which is found in large amounts in the brain, gastrointestinal tract, and genitourinary tract; CK-MM, which is found in skeletal and cardiac muscle; and the hybrid CK-MB, which is found primarily in cardiac muscle. In healthy adults, the predominant serum isoenzyme is CK-MM, and the concentration of CK-BB is very low (43, 52).

Using a radioimmunoassay, we have measured serum CK-BB in newly diagnosed, previously untreated patients with SCLC, and we correlated levels with extent of disease, sites of metastases, and tumor burden estimated by the number of clinically detectable metastatic sites. Serum CK-BB was also measured sequentially in patients receiving cytotoxic therapy and results compared with clinical responses. In addition, the expression of CK-BB in homogenates of SCLC cell lines derived from tumor specimens of patients was measured, and results were correlated with serum levels in these patients.

MATERIALS AND METHODS

Patient Population. Serum and tumor specimens were obtained from patients undergoing protocol staging procedures approved by appropriate Institutional Review Boards. All patients had a histologically confirmed diagnosis of SCLC. Patients routinely underwent the following pretreatment staging procedures: physical examination; fiberoptic bronchoscopy (with bronchial biopsy and cytological examination of washings); radionuclide scans of bone, liver, and brain; and bone marrow aspirate and biopsies. Liver biopsy was obtained in 49% of patients. Biopsies or fine-needle aspirates of enlarged lymph nodes, s.c. nodules, and pleural effusions were performed when clinically indicated.

Following staging procedures, patients with SCLC were designated as having limited disease (tumor confined to involved lung and regional lymph nodes) or extensive disease (tumor outside the above regions).
The number of distant metastatic organ systems involved with tumor was determined in all patients. Following staging, patients received intensive induction therapy with cyclophosphamide, methotrexate, and lomustine without dosage modification for hematological toxicity. Treatment thereafter varied but without significant therapy-related difference in survival. Details of protocol treatment have been reported elsewhere (11, 12).

Standard criteria for tumor response were used. A complete response required the disappearance of all clinical and pathological evidence of tumor in all known sites of disease; a partial response required a reduction of 50% or more in the sum of all measurable and evaluable tumor masses. Both were required to persist for a minimum of 4 weeks. Patients with less tumor reduction were considered to have no response. Survival was measured from Day 1 of chemotherapy.

CK-BB Determinations. Serum CK-BB was measured using a sensitive double-antibody radioimmunoassay (52). The antibody, raised against human brain CK-BB, did not cross-react with human CK-MM from muscle and reacted to a very low degree (about 1%) with CK-MB from cardiac muscle. In studies of 209 healthy adult volunteers, the mean serum CK-BB level was 3.4 ng/ml, the 95th percentile concentration was 6.2 ng/ml, and the coefficient of variation at the middle of the response range was approximately 5% within and 10% between assays (52). There was no relationship between age and CK-BB concentration. Minimally higher values were found in men, with mean values of 3.5 ng/ml in men and 3.1 ng/ml in women (44). For this study, serum levels greater than 10.0 ng/ml were considered elevated. This level was exceeded in only 4% of 25 heavy cigarette smokers and in only 6% of 108 individuals with nonmalignant diseases of the gastrointestinal or genitourinary tracts or of the breast (51). For serum CK-BB measurements, blood specimens were collected while patients were undergoing staging, and the serum was immediately separated after collection and stored at −70° prior to assay.

Statistical Methods. A χ² test for trend in proportions was used to evaluate the association between the number of metastatic sites and the presence of an elevated serum CK-BB level (3). The Mantel test (30) was used to examine the univariate effect between CK-BB levels and survival, and a stratified Mantel test was used to evaluate the joint effect on survival of CK-BB levels and the number of disease sites. All p values in this report correspond to 2-sided significance tests.

Cell Lines. Continuous cell lines of SCLC were established from biopsy material from 13 patients in this study. Details of culture methods, propagation, and characterization are presented elsewhere (9, 16). In brief, cultures were maintained in RPMI 1640 (Grand Island Biological Co., New York, NY) supplemented with 10% heat-inactivated fetal bovine serum (Grand Island Biological Co.). All cultures were continuous, clonal, and tumorigenic, and most were in culture for more than 6 months when tested. All cell lines have typical SCLC morphology and express high levels of the key APUD enzyme l-dopa decarboxylase (16). All cell lines were free of fibroblast contamination, and tests for Mycoplasma contamination were negative (Microbiological Associates, Inc., Bethesda, MD). For CK-BB analysis of cell cultures, cell pellets were collected, washed 3 times, and then resuspended in 1.0 ml phosphate-buffered saline (0.01 M phosphate buffer, pH 7.4, in 0.9% NaCl solution). Cells were disrupted in a Potter-Elvehjem tissue homogenizer, centrifuged (30,000 x g, 20 min), aliquoted immediately, and frozen at −70° until assayed. The CK-BB activity of the cell cultures was expressed as ng/mg protein. Protein determinations were performed by a one-reagent method (Bio-Rad, Richmond, CA) according to the manufacturer’s instructions.

RESULTS

Serum specimens for CK-BB measurements were obtained from 105 newly diagnosed patients with SCLC. Results are shown in Table 1. Overall, 27 patients (26%) had an elevated serum CK-BB (>10 ng/ml) [range, 11 to 522 ng/ml; median, 40.0 ng/ml; mean, 76.0 ± 19.2 (S.E.)]. Only 1 of 42 patients with limited-stage disease had an elevated serum CK-BB, while 26 of 43 (41%) patients with extensive-stage disease did.

When those patients with extensive stage were subgrouped according to the number of distant metastatic sites identified by pretreatment staging procedures, a significant association with the presence of an elevated serum CK-BB was observed at the p < 0.005 level. Six of 31 (19%) patients with 1 metastatic site; 6 of 16 (38%) patients with 2 sites; 6 of 8 (75%) patients with 3 sites; and 8 of 8 (100%) patients with 4 or more metastatic sites had an elevated level (Table 1). No marked association between the presence of metastatic SCLC in a specific site, such as brain, bone, or liver, and an elevated CK-BB was observed. In particular, of 11 patients with brain metastases at diagnosis, 7 had a CK-BB level <10 ng/ml.

Table 1

<table>
<thead>
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<th>Serum CK-BB activity in SCLC</th>
<th>No. with elevated serum CK-BB</th>
<th>% of patients with elevated CK-BB</th>
<th>Serum CK-BB (ng/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limited disease</td>
<td>42</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Extensive disease</td>
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<td></td>
<td></td>
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<tr>
<td>1 metastatic site</td>
<td>31</td>
<td>6</td>
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</tr>
<tr>
<td>2 metastatic sites</td>
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<tr>
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the number of metastatic site appeared to be independent variables which have significant correlation with survival.

**Serial CK-BB Determinations.** Repeat serum CK-BB determinations were performed in 33 patients 6 to 12 weeks from the onset of therapy when repeat staging procedures were routinely obtained. Serum CK-BB levels were correlated with the clinical response noted at that time. Of 12 patients who initially presented with extensive-stage disease and an elevated serum CK-BB, a fall in serum CK-BB was demonstrated at this time, in 10 to <10 ng/ml in from 2 to just above this value (Chart 1). Restaging procedures in these 12 patients revealed that all had responded to therapy, with 5 patients achieving a complete response and 7 patients attaining a partial response.

In a single patient, the repeat serum CK-BB had risen from 2.5 to 11.2 ng/ml (Chart 1). This patient did not respond to cytotoxic therapy and died soon after from progressive tumor growth, 4 months from the initial diagnosis. In 20 other patients who had a normal serum CK-BB level at diagnosis, the repeat CK-BB levels at restaging were also within the normal range. All of these patients had either a partial or a complete remission.

Multiple serum CK-BB determinations were obtained in 15 patients throughout the course of their disease. In 6 patients, the initially elevated serum CK-BB fell to <10 ng/ml (5 patients) or just above this level (one patient) with a clinical response to therapy. In all 6, repeat serum CK-BB determinations became elevated at the time of clinical tumor progression (Chart 2). In 5 patients, all of whom had a normal serum CK-BB level both at diagnosis and at the time of best clinical response, the serum CK-BB became elevated at the time of clinical relapse. In 4 other patients, 3 of whom had serum CK-BB levels at diagnosis <10 ng/ml, repeat serum CK-BB determinations at 6 weeks and throughout the following 2 years have all remained below 10 ng/ml. All of these 4 patients have remained free of disease and off all cytotoxic therapy for more than 18 months.

**Cell Cultures.** Continuous cell lines of SCLC were successfully established from 13 patients with extensive-stage disease included in this study. Among the 13 patients, serum CK-BB values varied from normal (5 patients) to elevated (8 patients). All cell lines expressed high levels of CK-BB compared to a variety of non-SCLC lung cancer cell lines (Table 2). Serum levels more closely related to the number of metastatic sites than to CK-BB activity of the corresponding patient’s cell line.

**DISCUSSION**

In this study of serum CK-BB determinations in 105 newly diagnosed patients with SCLC, we have shown that elevated levels were present in 26% of all patients. However, serum CK-BB levels were predominantly elevated in patients with extensive-stage disease (41%) compared to patients with limited-stage disease (2%).

In patients with extensive disease, an excellent correlation between the number of metastatic sites and the presence or absence of an elevated serum CK-BB was observed; 19% of all patients with a single metastatic site had an elevated serum CK-BB compared to 100% of all patients with 4 or more metastatic sites. In addition, independently of the number of metastatic sites, abnormal serum CK-BB levels had a significant adverse impact upon survival. Such prognostic significance of a biomarker that is independent of stage has not been demonstrated for CEA (29, 47); neurophysins (32); ACTH, ADH, and calcitonin (23); orNSE (10) in SCLC patients. However, a recent preliminary report in larger numbers of patients suggests that CEA levels may be of prognostic value that is independent of stage and performance status (38).

Sequential measurement of serum CK-BB in our patients receiving intensive combination chemotherapy also clearly indicated that serum CK-BB levels accurately reflected the clinically observed behavior of the tumor. Since only 11 patients were
studied from diagnosis through initial response to tumor progression and since intervals between specimen collection were variable, we have not sufficient data to estimate how often rising CK-BB levels represent the sole initial indication of tumor progression.

Serial measurements of neurophysins (32, 34), CEA (19, 47), NSE (10), ACTH and ADH (22), and calcitonin (22, 48) in SCLC patients receiving cytotoxic chemotherapy also demonstrate a good correlation between clinical response and serum levels of the markers in those patients who initially present with elevated marker values. However, there is no conclusive evidence that measurement of these markers provides useful clinical information which could not be obtained by systematic use of physical examination and routine staging procedures. Furthermore, the value of early detection of progressive SCLC remains debatable in view of the dismal response rate and survival with any form of treatment in patients who develop advancing disseminated SCLC on current aggressive therapy (33).

In addition to the brain, normal tissues known to contain substantial amounts of CK-BB are the intestine, prostate, testes, thyroid, kidney, lung, uterus, bladder, and stomach (43). The lack of association between the presence of brain metastases and an elevated serum CK-BB in our patients suggests that the brain itself is not the origin of the raised CK-BB levels. The excellent correlation between the number of metastatic sites clinically involved with tumor (tumor bulk) and elevated serum CK-BB and the expression of high levels of CK-BB in continuous cell cultures of SCLC both suggest that the source of elevated serum CK-BB levels in these patients is the tumor itself.

Elevated serum CK-BBs have been observed in a variety of human tumors (2, 13–15, 25, 26, 36, 39, 40, 42, 51). In a study of 366 patients with cancer, Zweig and Van Steirteghem (51) noted elevated serum CK-BB in 39 patients (11%). The highest fraction of patients with elevated serum CK-BB (>10 ng/ml) occurred in those with prostate cancer in whom 7 of 24 patients had raised levels. All 7 patients had Stage D prostate cancer. However, not all patients with Stage D cancer had elevated levels. In a study of 113 patients with breast cancer, Thompson et al. (42) noted that elevated levels (>3.0 ng/ml) correlated with extent of disease and clinical response to therapy. In addition, in this study, no correlation was observed between the presence of metastatic breast cancer in a specific site and the presence of an elevated serum CK-BB.

In a study by Rubery et al. (36), serum CK-BB was assayed in 1015 patients with histologically confirmed cancer. Elevated CK-BB levels (>3.0 ng/ml) were detected in 34% of patients with a variety of different tumors. In patients with breast cancer, tumor burden correlated with the degree of serum CK-BB elevations. In patients with a variety of lymphomas, 14 patients (34%) had moderately elevated CK-BB levels. Serial measurements in these patients correlated with response to therapy. In patients with bladder, prostate, testicular, and head and neck cancer, elevated CK-BB levels were more frequent in patients with metastatic disease than in those with localized disease.

Elevated serum CK-BB levels have also been reported previously in patients with lung cancer. Coolen et al. (13), in a study of sera from 39 patients with cancer, noted elevated serum CK-BB in 12 of 15 patients with SCLC. Only one patient had CNS metastasis. In addition, extracts of tumor tissue from autopsies of 2 patients with SCLC revealed elevated CK-BB activity. Increasing levels of CK-BB were noted in 2 patients with progression of their disease. In the report by Rubery et al. (36), serum CK-BB was elevated in 41% of 95 patients with bronchogenic carcinoma. No data by histological cell type were reported.

SCLC, both in vivo and in vitro, is associated with the production of a large number of biological compounds including ACTH, ADH, calcitonin, neurophysins, and CEA. However, none of these substances is produced exclusively by SCLC tumor cells, and some are found in large quantities in the blood and urine of patients with a variety of nonneoplastic disorders. Unlike these other hormones and secretory products of SCLC which have considerable heterogeneity of expression both in vivo and in vitro, high concentrations of CK-BB can be demonstrated in all cell lines of SCLC (17). These data suggest that screening of established cell lines for specific markers may provide a means of detecting biomarkers useful clinically in the treatment of that same tumor. Previously, we have shown that NSE, a neuronal glycolytic enzyme, was significantly elevated in cell lines of SCLC but not in non-SCLC cell lines (31). Evaluation of serum NSE levels in patients with SCLC revealed elevated levels (>12.0 ng/ml) in 69%, including 15 of 38 patients (47%) with limited-stage disease and 87% of 56 patients with extensive-stage disease (10). An excellent correlation between serum NSE levels and tumor burden and response to therapy was also observed. Once stage of disease was accounted for, however, there was no relationship between NSE values and response or survival.

Although serum measurement of CK-BB may be of limited value in the initial diagnosis of patients with SCLC, immunohistochemical staining or biochemical analysis of lung tumors, especially anaplastic ones, for CK-BB may clearly differentiate those of SCLC origin from the other major histological subtypes (18, 37). Such differentiation would have a major impact on selection of therapy. Because high levels of creatine kinase and its substrate are a mechanism for the rapid regeneration of ATP levels depleted during muscle contraction, the elevated levels of CK-BB in SCLC, in contrast to non-SCLC lung cancer cells, suggest that the energy requirements of SCLC may be markedly different from those of non-SCLC. Such differences, if present, could potentially be exploited in the treatment of the different types of lung cancer.
REFERENCES


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