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Elevated Serum Creatine Kinase BB Levels in Patients with Small Cell Lung Cancer[1]

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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[3] 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.

Creatine kinase (ATP-creatine *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).

[1] The opinions expressed herein are those of the authors and are not to be construed as reflecting the views of the Navy Department, of the Naval Service at large or of the Department of Defense.

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[3] The abbreviations used are: SCLC, small cell lung cancer; CK-BB, BB isoenzyme of creatine kinase; APUD, amine precursor uptake and decarboxylation; NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; ACTH, adrenocorticotrophic hormone; ADH, antidiuretic hormone; CK-MM, MM isoenzyme of creatine kinase; CK-MB, MB isoenzyme of creatine kinase.

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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 χ^2 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, clonable, 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 × 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.

Serum CK-BB and Survival. The median survival of all patients was 12 months. Among all patients with a normal serum CK-BB (<10 ng/ml) at diagnosis, survival was significantly better than for those with an elevated CK-BB ($p < 0.001$) (median of 13 months *versus* 5 months). However, as reported previously (27), survival was significantly affected by the number of metastatic sites detected at diagnosis. Among patients with 4 or more metastatic sites, survival was significantly inferior to that among patients with limited-stage disease, or those with 1, 2, or 3 sites of metastatic disease ($p < 0.001$ for each comparison), and there was a significant trend for patients to have a progressively shorter survival as the number of metastatic sites of disease increased.

Because the number of metastatic sites and the serum CK-BB levels were identified as important prognostic factors, a stratified Mantel analysis was carried out to further evaluate their joint relationship with survival. After adjustment for the number of metastatic sites, survival for patients with a normal CK-BB was significantly better than for those with an elevated CK-BB ($p = 0.014$). In addition, after accounting for the CK-BB level, the number of metastatic sites clinically detected also significantly affected survival ($p < 0.005$). Thus, serum CK-BB and

Table 1
Serum CK-BB activity in SCLC

	No. of patients	No. with elevated serum CK-BB	% of patients with elevated CK-BB	Serum CK-BB (ng/ml serum)		
				Mean ± S.E.	Median	Range
Limited disease	42	1	2	3.7 ± 0.50	3.0	0.7–21
Extensive disease						
1 metastatic site	31	6	19	15.5 ± 6.39	4.7	0.4–185
2 metastatic sites	16	6	38	43.9 ± 32.06	8.2	0.7–522
3 metastatic sites	8	6	75	43.0 ± 12.87	42	2.7–108.6
≥4 metastatic sites	8	8	100	82.9 ± 36.57	35	14–299
Total	105	27	26			

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

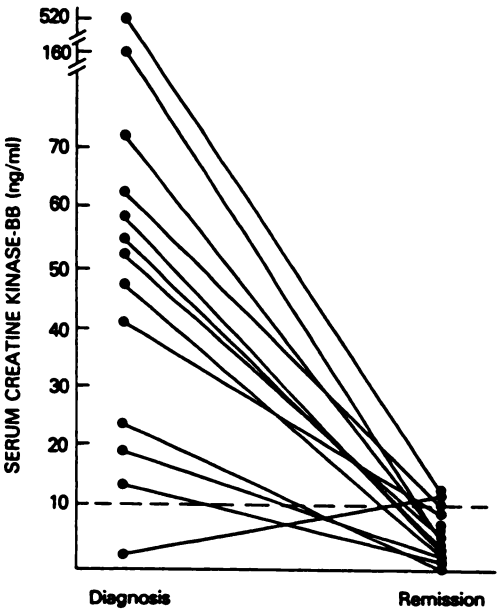

Chart 1. Serum CK-BB determinations in 13 patients at diagnosis and at the end of induction chemotherapy when repeat staging procedures were routinely performed (6 to 12 weeks from onset of therapy). Among 12 patients who initially had an elevated CK-BB and who responded to therapy, repeat CK-BB determinations demonstrated that 10 had fallen to within the normal range (<10 ng/ml) and 2 had come just above it. In one patient, who initially had a CK-BB level of 2.5 ng/ml, no response to therapy was obtained, and repeat CK-BB at 6 weeks revealed an elevated level (11.2 ng/ml). In 20 other patients, all of whom responded to therapy, serum CK-BB was within normal limits at both diagnosis and at restaging (not shown).

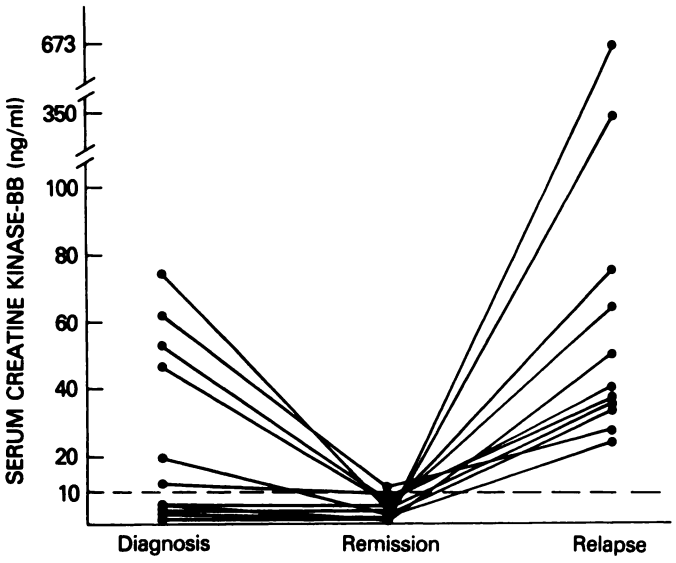

Chart 2. Sequential serum CK-BB determinations in 11 SCLC patients at diagnosis, at initial clinical response, and at relapse from cytotoxic therapy.

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); or NSE (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

Table 2
CK-BB activity in established cell lines of SCLC

Patient	No. of metastatic sites	Serum CK-BB (ng/ml)	Cell line morphology	Cell line CK-BB (ng/ml)
NCI-H230	1	11.7	SCLC	3,783
NCI-H220	1	3.3	SCLC	3,963
NCI-H182	1	3.9	SCLC	9,856
NCI-H298	1	3.0	SCLC	6,565
NCI-H187	2	8.1	SCLC	8,234
NCI-H301	2	11.2	SCLC	3,730
NCI-H249	2	10.0	SCLC	17,555
NCI-H123	2	18.2	SCLC	5,993
NCI-H390	2	47.6	SCLC	3,323
NCI-H209	4	299.0	SCLC	3,823
NCI-H285	4	17.5	SCLC	10,733
NCI-H289	4	185.0	SCLC	12,740
NCI-H250	4	34.0	SCLC	3,861

	Total	Mean
SCLC	13	7,531 (3,323–17,555)[a]
Non-SCLC[b]	12	103 (0–443)

[a] Numbers in parentheses, range.
[b] Includes 5 adenocarcinomas, 4 large cell carcinoma, one squamous cell carcinoma, and 2 mesotheliomas [from the paper of Gazdar *et al.* (17)].

studied from diagnosis through initial response to tumor progression and since intervals between specimen collection were variable, we do not have 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

1. Abeloff, M. D., Trump, D. L., and Baylin, S. B. Ectopic adrenocorticotrophic (ACTH) syndrome and small cell carcinoma of the lung: assessment of clinical implications in patients on combination chemotherapy. Cancer (Phila.), *48:* 1082–1087, 1981.
2. Aleyassine, H., and MacIsaac, S. G. The diagnostic significance of serum creatine kinase-BB isoenzyme in adenocracinoma of prostate. Clin. Biochem., *13:* 109–112, 1980.
3. Armitage, P. Statistical Methods in Medical Research. Oxford, England: Blackwell Scientific Publications, 1971.
4. Baylin, S. B., Abeloff, M. D., Goodwin, G., Carney, D. N., and Gazdar, F. Activities of L-dopa decarboxylase and diamine oxidase (histaminase) in lung cancers and decarboxylase as a marker for small (oat) cell cancer in culture. Cancer Res., *40:* 1990–1994, 1980.
5. Baylin, S. B., Weisburger, W. R., Eggleston, J. C., Mendelson, G., Beaven, M. A., Abeloff, M. D., and Ettinger, D. S. Variable content of histaminase, L-dopa decarboxylase and calcitonin in small-cell carcinoma of the lung. N. Engl. J. Med., *299:* 105–110, 1978.
6. Bensch, K., Corrin, B., Parients, R., and Spencer, H. Oat cell carcinoma of the lung, its origin and relationship to bronchial carcinoids. Cancer (Phila.), *22:* 1163–1177, 1976.
7. Cancer Statistics 1980. CA, *31:* 13–28, 1981.
8. Carney, D. N., Broder, L. E., Edelstein, M., Gazdar, A. F., Hansen, M., Havemann, K., Matthews, M. J., Sorenson, G. D., and Vindelov, L. Experimental studies of the biology of human small cell lung cancer. Cancer Treat. Rep., *67:* 27–36, 1983.
9. Carney, D. N., Bunn, P. A., Gazdar, A. F., Pagan, J., and Minna, J. D. Selective growth of small cell carcinoma of the lung obtained from patient biopsies in serum-free hormone supplemented medium. Proc. Natl. Acad. Sci. USA, *78:* 3185–3189, 1981.
10. Carney, D. N., Marangos, P. J., Ihde, D. C., Cohen, M. H., Bunn, P. A., Minna, J. D., and Gazdar, A. F. Serum neuron-specific enolase: a marker for disease extent and response to therapy of small-cell lung cancer. Lancet, *1:* 583–585, 1982.
11. Cohen, M. H., Creaven, P. J., Fossieck, B. E., Broder, L. E., Selawry, O. S., Johnston, A. V., Williams, C. L., and Minna, J. D. Intensive chemotherapy in small cell bronchogenic carcinoma. Cancer Treat. Rep., *61:* 349–354, 1977.
12. Cohen, M. H., Ihde, D. C., Bunn, P. A., Fossieck, B. E., Matthews, M. J., Shackney, S. E., Johnston-Early, A., Makuch, R., and Minna, J. D. Cyclic alternating combination chemotherapy for small cell bronchogenic carcinoma. Cancer Treat. Rep., *63:* 163–170, 1979.
13. Coolen, R., Pragay, D., Nosanchuk, J., and Belding, R. Elevation of brain-type creatine kinase in serum from patients with carcinoma. Cancer (Phila.), *44:* 1414–1418, 1979.
14. Forman, D. L. The significance of creatine kinase (CKBB) in metastatic cancer of the prostate. Ann. Clin. Lab. Sci., *9:* 333–338, 1979.
15. Ganz, P., Potter, R., Figlin, R., and Shell, W. Creatine kinase-BB (CK-BB): a tumor marker for active metastatic cancer. Proc. Am. Assoc. Cancer Res., *22:* 343, 1981.
16. Gazdar, A. F., Carney, D. N., Russell, E. K., Sims, H. L., Baylin, S. B., Bunn, P. A., Guccion, J., and Minna, J. D. Establishment of continuous clonable cultures of small-cell carcinoma of the lung which have amine precursor uptake and decarboxylation cell properties. Cancer Res., *40:* 3502–3507, 1980.
17. Gazdar, A. F., Zweig, M. H., Carney, D. N., Van Steirteghem, A. C., Bayin, S. B., and Minna, J. D. Levels of creatine kinase and its BB isoenzyme in lung cancer specimens and cultures. Cancer Res., *41:* 2773–2777, 1981.
18. Goslin, R., O'Brien, J., Skarin, A. T., and Zamchek, N. Immunocytochemical staining for CEA in small cell carcinoma of lung predicts clinical usefulness of the plasma assay. Cancer (Phila.), *52:* 301–306, 1983.
19. Goslin, R. H., Skarin, A. T., and Zamchek, N. Carcinoembryonic antigen: a useful monitor of therapy of small cell lung cancer. J. Am. Med. Assoc., *246:* 2173–2176, 1981.
20. Gropp, C., Havemann, K., and Scheuer, A. Ectopic hormones in lung cancer patients at diagnosis and during therapy. Cancer (Phila.), *46:* 347–354, 1980.
21. Hallgren, R., Nou, E., and Lundqvist, G. Serum B_2-microglobulin in patients with bronchial carcinoma and controls. Cancer (Phila.), *45:* 780–785, 1980.
22. Hansen, M., Hammer, M., and Hummer, L. ACTH, ADH and calcitonin concentrations as markers of response and relapse in small-cell carcinoma of the lung. Cancer (Phila.), *45:* 2062–2067, 1980.
23. Hansen, M., Hammer, M., and Hummer, L. Diagnostic and therapeutic implications of ectopic hormone production in small cell carcinoma of the lung. Thorax, *35:* 101–106, 1980.
24. Hansen, M., Hansen, H. H., Hirsch, F. R., Arends, J., Christensen, J. D., Christensen, J. M., Hummer, L., and Kuhl, C. Hormonal polypeptides and amine metabolites in small cell carcinoma of the lung, with special reference to stage and subtypes. Cancer (Phila.), *45:* 1432–1437, 1980.
25. Hoag, G., Amies, D., and Colquhoun, B. The production of creatine kinase isozyme BB in sera of a patient with prostatic carcinoma and in tumor homogenates. Clin. Biochem., *11:* 38–41, 1978.
26. Hoag, G., Franks, C., and DeCoteau, W. Creatine kinase isoenzymes in serum of patients with cancer of various organs. Clin. Chem., *24:* 1654, 1978.
27. Ihde, D. C., Makuch, R. W., Carney, D. N., Bunn, P. A., Cohen, M. H., Matthews, M. J., and Minna J. D. Prognostic implications of stage of disease and sites of metastases in patients with small cell carcinoma of the lung treated with intensive chemotherapy. Am. Rev. Respir. Dis., *123:* 500–507, 1981.
28. Kornguth, S. E, Klein, R., Appen, R., and Choate, J. Occurrence of anti-retinal ganglion cell antibodies in patients with small cell carcinoma of the lung. Cancer (Phila.), *50:* 1289–1293, 1982.
29. Lokich, J. L. Plasma CEA levels in small cell lung cancer: correlation with stage, distribution of metastases, and survival. Cancer (Phila.), *50:* 2154–2156, 1982.
30. Mantel, N. Evaluation of survival data and two new rank-order statistics arising in its consideration. Cancer Chemother. Rep., *50:* 163–170, 1966.
31. Marangos, P., Gazdar, A., and Carney, D. Neuron specific enolase in human small cell carcinoma cultures. Cancer Lett., *15:* 67–71, 1982.
32. Maurer, L. H., O'Donnell, J. F., Kennedy, S., Faulkner, C. S., Rist, K., and North, W. G. Human neurophysins in carcinoma of the lung: relation to histology, disease stage, response rate, survival, and syndrome of inappropriate antidiuretic hormone secretion. Cancer Treat. Rep., *67:* 971–976, 1983.
33. Morstyn, G., Ihde, D. C., Lichter, A. S., Bunn, P. A., Carney, D. N., Glatstein, E., and Minna, J. D. Small cell lung cancer 1973–1983: early progress and recent obstacles. Int. J. Radiat. Oncol. Biol. Phys., *10:* 515–539, 1984.
34. North, W., Maurer, H., Valtin, H., and O'Donnell, J. Human neurophysins as potential tumor markers for small cell carcinoma of the lung: application of specific radioimmunoessays. J. Clin. Endocrinol. Metab., *51:* 892–890, 1980.
35. Pettengill, O., Sorenson, G. Wurster-Hill, D., Curphey, T. J., Noll, W. W., Cate, C. C., and Maurer, L. H. Isolation and growth characteristics of continuous cell lines from small cell carcinoma of the lung. Cancer (Phila.), *45:* 906–918, 1980.
36. Rubery, E. D., Doran, J. F., and Thompson, R. J. Brain-type creatine kinase BB as a potential tumor marker: serum levels measured by radioimmunoessay in 1015 patients with histologically confirmed malignancies. Eur. J. Cancer Clin. Oncol., *18:* 951–956, 1982.
37. Said, J., Nash, G., Tepper, G., and Banks-Schlegel, S. Keratin proteins and carcinoembryonic antigen in lung carcinoma: an immunoperoxidase study of fifty-four cases, with ultrastructural correlations. Hum. Pathol., *14:* 70–76, 1983.
38. Sculler, J. P., Feld, R., Evans, W. K., Shepherd, F. A., De Boer, G., Malkin, D. G., and Malkin, A. CEA: a useful prognostic marker for small cell lung cancer. Proc. Am. Assoc. Cancer Res., *25:* 158, 1984.
39. Silverman, L. M., Caruso, L. M., Irwin, L. E., Kitzman, M., and Pincus, F. E. Creatine kinase BB isozyme activity in bone-marrow serum. Clin. Chem., *24:* 1423–1425, 1978.
40. Silverman, L. M., Dermer, G. B., Zweig, M. H., Van Steirteghen, A. C., and Tokes, Z. A. Creatine kinase BB: a new tumor-associated marker. Clin. Chem., *25:* 1432–1435, 1979.
41. Sorenson, G. D., Pettengill, O. S., Brink-Johnson, T., Cate, C. C., and Maurer, L. H. Hormone production by cultures of small cell carcinoma of the lung. Cancer (Phila.), *47:* 1289–1296, 1981.
42. Thompson, R. J., Rubery, E. D., and Jones, H. M. Radioimmunoassy of serum creatine kinase-BB as a tumor marker in breast cancer. Lancet, *2:* 673–675, 1980.
43. Tsung, S. W. Creatine kinase isoenzyme patterns in human tissue obtained at surgery. Clin. Chem., *22:* 173–175, 1976.
44. Van Steirteghem, A. C., Robertson, E. A., and Zweig, M. H. Distribution of serum concentrations of creatine kinase MM and BB isoenzymes measured by radioimmunoessay. Clin. Chim. Acta, *93:* 25–28, 1979.
45. Waalkes, T. P., Abeloff, M. D., Ettinger, D. S., Woo, K. B., Gehrke, C. W., Kuo, K. C., and Borek, E. Biological markers and small cell carcinoma of the lung: a clinical evaluation of urinary ribonucleosides. Cancer (Phila.), *50:* 2457–2464, 1982.
46. Waalkes, T. P., Abeloff, M. D., Ettinger, D. S., Woo, K. B., Kuo, K. C., and Gehrke, C. W. Serum protein-bound carbohydrates and small cell carcinoma of the lung: correlations with extent of disease, tumor burden, survival, and clinical response categories. Cancer (Phila.), *52:* 131–139, 1983.
47. Waalkes, T. P., Abeloff, M. D., Woo, K. C., Ettinger, D. S., Ruddon, R. W., and Aldenderfer, P. Carcinoembryonic antigen for monitoring patients with small cell carcinoma of the lung during treatment. Cancer Res., *40:* 4420–4427, 1980.
48. Wallach, S. R., Royston, I., Taetle, R., Whol, H., and Deftos, L. J. Plasma calcitonin as a marker of disease activity in patients with small cell carcinoma of the lung. J. Clin. Endocrinol. Metab., *53:* 602–610, 1981.
49. Woo, K. C., Waalkes, T. P., Abeloff, M. D., Ettinger, D. S., McNitt, K. L., and Gehrke, C. W. Multiple biologic markers in the monitoring of treatment for patients with small cell carcinoma of the lung: the use of serial levels of plasma CEA and serum carbohydrates. Cancer (Phila.), *48:* 1633–1642, 1981.
50. Yalow, R. S., Eastridge, C. E., Higgins, G., and Wolf, J. Plasma and tumor ACTH in carcinoma of the lung. Cancer (Phila.), *44:* 1789–1792, 1979.
51. Zweig, M. H., and Van Steirteghem, A. C. Assessment by radioimmunoessay of serum creatine kinase BB (CK-BB) as a tumor marker: studies in patients with various cancers and a comparison of CK-BB concentrations to prostate acid phosphatase concentrations. J. Natl. Cancer Inst., *66:* 859–862, 1981.
52. Zweig, M. H., Van Steirteghem, A. C., and Schechter, A. Radioimmunoessay of creatine kinase isoenzymes in human serum: isoenzyme BB. Clin. Chem., *24:* 422–428, 1978.

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