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Early Detection of Response in Small Cell Bronchogenic Carcinoma by Changes in Serum Concentrations of Creatine Kinase, Neuron Specific Enolase, Calcitonin, ACTH, Serotonin and Gastrin Releasing Peptide

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Abstract—*Creatine kinase (CK-BB), neuron specific enolase (NSE), ACTH, calcitonin, serotonin and gastrin releasing peptide (GRP) were measured in serum or plasma before and immediately after initiation of treatment in patients with small cell lung cancer (SCC). Pretherapeutic elevated concentrations of CK-BB were found in 82% of extensive disease patients and in 50% of patients with local disease. NSE was raised in 72% with extensive disease versus 14% of patients with local disease. Calcitonin and ACTH were raised in 27% and 28%, respectively, in all patients without significant difference between extensive and local disease patients. Serotonin was generally overall elevated in 10% and GRP in 7% but elevations were seen only in patients with extensive disease. Out of the four most frequently elevated substances at least one marker was elevated in 80% of all the patients, including 91% in extensive stage patients and 71% in limited stage patients.*

Frequent initial monitoring of the substances showed an increase in the concentrations of pretherapeutic elevated CK-BB and NSE on day 1 or 2 followed by a sharp decrease within 1 week. These changes were correlated to objective clinical response determined within 4–8 weeks.

The results indicate that serum CK-BB and NSE are potential markers for SCC at the time of diagnosis and that changes in the concentrations during the first course of cytostatic therapy are promising as biochemical tests for early detection of response to chemotherapy.

INTRODUCTION

PRODUCTION of peptide hormones is well documented in small cell lung cancer (SCC). The incidence of elevated concentrations in untreated patients is about 30% for ACTH and ADH and about 60% for calcitonin [1]. A number of other peptides and amines were investigated, but significantly elevated concentrations were found in only a few cases [1].

Recently it was demonstrated in *in vitro* studies that the neuronal enzymes neuron specific enolase (NSE) and creatine kinase BB isoenzyme (CK-BB), and also the peptide bombesin or its mammalian counterpart gastrin releasing peptide (GRP) are produced in almost all cell lines from SCC [2–4]. These findings have renewed the interest in evaluating these substances for their clinical utility as tumor markers.

In a number of studies NSE has been found to be elevated in 60–85% of untreated patients with higher incidences in patients with extensive disease (ED) [5–11]. In two studies CK-BB was found to be elevated in about 40% of untreated ED patients [3, 12] while GRP has not been demonstrated in blood specimens in a significant number of patients [13].

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The aim of the present study was to investigate the potential markers at the time of diagnosis and to evaluate changes in concentrations of these substances after initiation of chemotherapy for SCC.

PATIENTS AND METHODS

The investigation included 32 consecutive patients with confirmed SCC (WHO II), who accepted participation after admission to the department of Internal Medicine C, Bispebjerg Hospital, Copenhagen from September 1983 to June 1984.

All patients underwent routine investigations for staging of the disease, including physical examination, chest X-ray, bronchoscopy, biopsy of the liver, bilateral bone marrow biopsy with aspiration and histo- or cytologic examination of other suspected lesions. Patients with lesions including only one lung, mediastinum and supraclavicular lymph nodes were defined as having limited disease. Patients with documented disease outside these regions were classified as extensive disease patients. All patients received combination chemotherapy according to controlled trials, which in the study period consisted of four or five agents [14]. Radiotherapy or surgery was not part of the initial therapy. X-Rays of the lungs were performed at least every 4 weeks.

All patients had blood sampling performed before start of treatment. In 17 patients sampling was repeated on days 1, 2, 4, 8, 12, 15, 21 and 28. Concentrations of CK-BB, NSE, calcitonin and serotonin were measured in serum samples, ACTH and GRP in plasma samples. The test tubes were immediately placed on ice and centrifuged within 1 h and plasma or serum were kept frozen at −20°C until the analyses were performed. All substances were measured by radioimmunoassay techniques as previously described: CK-BB by Urdal *et al.* [15], NSE by Paus and Risberg [16], calcitonin by Schifter [17], ACTH by Fenger [18], serotonin by Engbaek and Voldby [19] and GRP by Holst [20, 21].

RESULTS

Incidence of elevated levels

The frequency of pretherapeutic elevated concentrations of CK-BB, NSE, ACTH, calcitonin and GRP are presented in Table 1. CK-BB was elevated in 82% of extensive disease patients versus 50% of limited disease patients. NSE was increased in 72% of extensive versus 14% of limited disease patients. ACTH and calcitonin were elevated in 28% and 27%, respectively, without significant differences between extensive and limited disease patients. Serotonin was elevated in 23% and GRP in 16% of extensive disease patients.

Concentrations of all the above mentioned substances were markedly increased only in a few patients. Two patients had pretherapeutic levels of CK-BB of 45 and 125 μg/l, respectively, while the other 14 patients with elevated levels had slightly increased concentrations ranging from 3.6 to 7.6 μg/l. The normal upper reference limit was 3.5 μg/l. NSE was moderately increased with a median of 27, range 17–54 μg/l. The normal upper reference limit was 15 μg/l. The reference range for calcitonin was 20–75 pmol/l. Increased concentrations ranged from 78 to 240 with a median of 99 pmol/l. Except for one patient with marked elevation (340 pmol/l) ACTH was only slightly increased above the normal upper reference limit of 36 pmol/l. Serotonin was markedly increased above the upper limit of 1800 nmol/l in three of 13 patients. Another three patients had concentrations below the lower reference limit of 250 nmol/l. Although the reference limits of GRP has not been exactly defined only two patients had high concentrations of 36 and 90 pmol/l respectively, while the remaining 26 patients had concentrations below 10 pmol/l with a median of 5 ranging from 0 to 10.

Tumor marker panel

In 25 patients the pretherapeutic values of the four most frequently elevated substances were evaluated as a marker panel. CK-BB was the most frequently elevated single marker (64%). NSE alone was elevated in only one patient in whom CK-BB was not elevated. Including also calcitonin and ACTH sucessively, the incidence of at least one elevated marker increased to 72% and 80%, respectively.

Frequent monitoring after initiation of treatment

In 17 consecutive patients blood sampling was performed on days 0, 1, 4, 8, 12, 15, 21 and 28. For each of the four markers, CK-BB (12/17), NSE (6/17), ACTH (5/17) and calcitonin (5/17) the course of the concentrations in patients with elevated pretherapeutic values are presented as ratio of the initial values (Figs. 1–4). Within days 1 and 2 the concentrations of CK-BB and NSE increased to a median of 50% above the pretreatment level (range 20–100% and 10–70%, respectively), followed by a decrease on days 4–8 to a median level of 40% of the pretreatment concentrations. In most cases the level remained at these low levels during the following period of 28 days. The changes in actual serum/plasma concentrations from one selected patient are shown in Fig. 5.

Three of five patients with initial normal concentrations of CK-BB and 1 of 11 with initial normal concentration of NSE showed similar changes with an increase above upper limit followed by a decrease

Table 1. Frequency of elevated serum/plasma concentrations of individual markers in untreated patients with small cell carcinoma

Substance	Number of patients with raised levels Total	%	Extensive	%	Limited	%
CK-BB	16/25	64	9/11	82	7/14	50
NSE	10/25	40	8/11	72	2/14	14
ACTH	9/32	28	3/13	23	6/19	32
Calcitonin	8/29	27	4/13	31	4/16	25
Serotonin	3/29	10	3/13	23	0/16	0
GRP	2/28	7	2/12	16	0/16	0

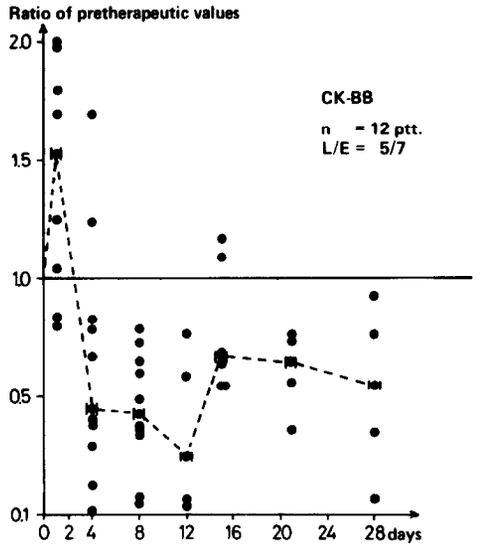

Fig. 1. Changes in serum CK-BB concentrations during first cytostatic course in patients with SCC (median values ——).

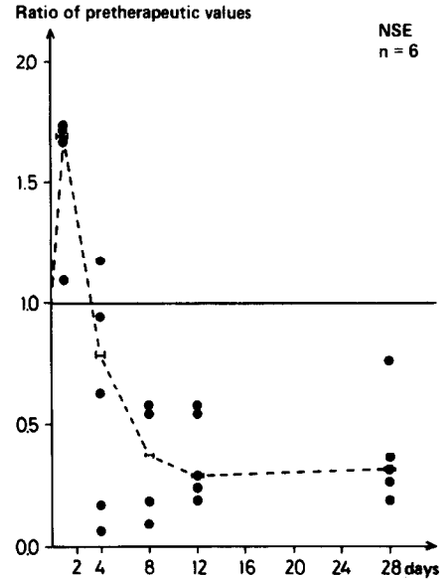

Fig. 2. Changes in serum NSE concentrations during first cytostatic course in patients with SCC (median values ——).

within 1 week. For ACTH and calcitonin no significant elevation was observed initially and the subsequent decrease was less pronounced. The two patients with pretreatment high concentrations of GRP demonstrated a constant decrease to values below 10 pmol/l within 1 week.

The course of the marker changes correlate to tumor regression. In 11 of the 12 patients with pretreatment elevation of CK-BB a tumor remission of 50% or more was seen within 8 weeks. In eight of these 11 patients the concentrations of CK-BB initially increased within 2 days followed by a decrease within the subsequent week. In two patients the concentration of CK-BB decreased without the detection of an initial elevation and in one patient the concentrations remained slightly elevated. One patient showed the characteristic course without demonstrating objective remission according to WHO criteria (Table 3). With regard to NSE all six patients who had pretherapeutic elevated levels followed by an increase and subsequent fall were responders.

DISCUSSION

CK-BB has only been investigated as a possible tumor marker in SCC in two prior studies. Compared to the study by Carney *et al.* [12] CK-BB is found in the present study to be more frequently elevated in untreated SCC patients, the incidences being 82% versus 41% in extensive disease patients and 50% versus 2% in patients with limited disease. Different assays and also the use of different reference limits may contribute to some of the differences. The patient populations seem to be comparable.

The pretherapeutic incidence of increased NSE in 72% of extensive disease patients found in this study is not significantly different from the incidences found by other investigators [5–11], who demonstrated elevated concentrations in ED patients ranging fom 78% to 98%. On the contrary, the incidences of elevated NSE in limited disease patients were significantly higher in those studies ranging from 37% to 71% versus 14% in the present study. As a higher proportion of patients with

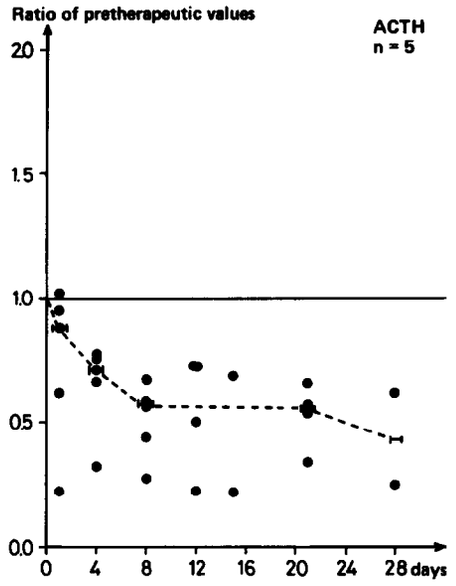

Fig. 3. Changes in plasma ACTH concentrations during first cytostatic course in patients with SCC (median values ——).

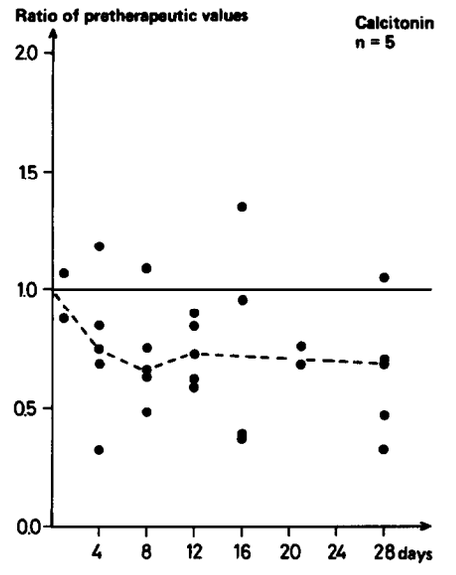

Fig. 4. Changes in serum calcitonin concentrations during first cytostatic course in patients with SCC (median vlaues ——).

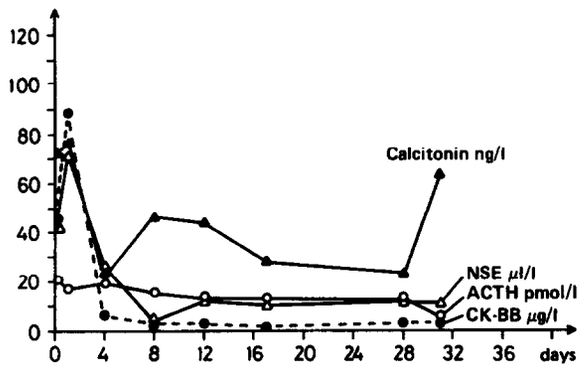

Fig. 5. Changes in serum/plasma concentrations of CK-BB (●—●), NSE (△ ——△), ACTH (○—○) and calcitonin (▲—▲) in one patient after start of chemotherapy for small cell lung cancer.

limited disease are represented in our study the overall incidence of 40% for NSE is lower than previously demonstrated.

Interestingly enough, GRP was found to be elevated in only two of 28 patients. This substance is in *in vitro* investigations produced in the majority of SCC cell lines and also in tumor tissue samples [4, 13]. In the latter study none of five patients with known high amounts of GRP in tumor tissue had detectable concentrations in plasma. The reason for this may be due to a very fast degradation of the GRP peptide in peripheral blood.

The incidence of elevated serum calcitonin of 27% is lower than previously found in an investigation of a similar population [1], but another assay has been applied in this study. In general, great variations in pretherapeutic values of calcitonin have been observed in several studies [22] ranging from 25% to 76% [23, 24].

ACTH has been demonstrated to be elevated in about 30% of patients in several investigations [22], which was also found in the present study.

To our knowledge the present finding of increased serotonin in 23% of the patients with extensive disease has not been observed before.

The four most frequently elevated substances in this study were also evaluated with regard to a marker panel. The incidence of at least 1 elevated marker increased from 64% using CK-BB alone to 80% using CK-BB, NSE, CT and ACTH together (Table 2). An incidence of 91% was found in ED patients using CK-BB and NSE or all substances together. Previously, a frequency of at least one elevated marker in a panel consisting of calcitonin, CEA and HCG-beta of 50% were found [23], of 78% using ACTH, calcitonin, PTH and HCG-beta [25] and an incidence of 84% using ACTH, ADH and calcitonin [22, 26].

In previous studies the serum concentration of NSE has been shown to correlate to the clinical course of the disease. Thus, a decrease observed a few weeks after initiation of treatment was related to response and subsequent elevation before or at the time of clinical relapse has been described in the studies on NSE [5–11]. Similar changes in serum concentrations of CK-BB related to the clinical course have been described [12]. Also high values of NSE were found on day 3 during the first 3-day course of cytostatic infusion for SCC primarily in responding patients [10].

In the present study changes in serum concentrations of CK-BB and NSE were demonstrated to be initial increases of the substances to about 100% on day 1 followed by a sharp decrease substantially below pretreatment levels within 1 week. These courses of the concentrations indicate a therapeutic effect with lysis of tumor cells. This explanation is supported by a demonstrated increase of serum

Table 2. Incidence of at least one elevated substance in a marker panel

Substance	Number of patients with raised levels Total	%	Extensive	%	Limited	%
CK-BB	16/25	64	9/11	82	7/14	50
CK-BB + NSE	17/25	68	10/11	91	7/14	50
CKBB + NSE + CT	18/25	72	10/11	91	8/14	57
CKBB + NSE + CT + ACTH	20/25	80	10/11	91	10/14	71

Table 3. Pretherapeutic increase of CK-BB versus response

Patient No.	CK-BB	Response	Duration in months
1	+	CR*	10.5
2	+	CR*	13
3	−	PR	2
4	+	CR*	8
5	+	NC*	5.5
6	−	NC	5.5
7	+	CR	18+
8	+	PR*	6
9	−	NE*	18+
10	+	PR*	4
11	+	CR*	18+
12	−	PR*	11
13	+	PR	4
14	−	PR*	8
15	+	PR	2
16	+	PR	11
17	+	PR*	5

*Initial increase followed by decrease of CK-BB.

phosphate and potassium within 1 or 2 days after administration of chemotherapy to acute lymphocytic leukemia and Burkitt's lymphoma respectively [27,28]. In a case report initial increase of hormonal substances from an APUDoma responding to therapy has been described [29]. Measurements of alfa-fetoprotein and HCG-beta during treatment of testicular cancer shows similar changes in responding patients.

It might be a major advantage to have reliable tumor markers for small cell lung cancer with respect to an early detection of response to therapy either as a prediction of prognosis and selection of treatment strategy for individual patients or as a tool for evaluation of the activity of new cytostatic agents in phase II trials.

Thus, the results of the present study indicate that serum CK-BB and NSE are potential markers for small cell lung cancer at the time of diagnosis. Changes in concentrations of the substances within 1 week after initiation of treatment are promising for the development of biochemical tests for early detection of response to chemotherapy. Further investigations on this subject are of considerable interest.

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