Potential Utility of Serum Neuron-specific Enolase Levels in Small Cell Carcinoma of the Lung


ABSTRACT

To assess the value of neuron-specific enolase (NSE) as a possible biomarker of small cell lung cancer, serum levels were determined by radioimmunoassay in 93 newly diagnosed untreated patients and were compared to the NSE levels of 20 healthy adult controls ($9.6 \pm 0.7$ (S.E.) ng/ml). Serum NSE was elevated ($>20$ ng/ml) in 73% of all patients including 23 of 39 (59%) with limited-stage disease and 45 of 54 (83%) with extensive-stage disease. The mean serum NSE was significantly higher in extensive-stage disease (94.5 $\pm 13.8$ ng/ml) compared to the mean value for limited-stage disease (33.7 $\pm 4.7$ ng/ml) ($p < 0.001$). NSE was elevated in all patients with three or more sites of metastatic disease. Serial NSE determinations were obtained on 57 small cell lung cancer patients. NSE levels fell in 40 of 50 (80%) of patients responding to treatment, increased in 5 of 7 (71%) of patients with progressive disease, and increased in 30 of 35 (86%) of patients who relapsed. A persistent increase in serum NSE occurred as many as 12 weeks before the clinical recognition of relapse in 15 of 23 (65%) of patients for whom adequate serial NSE data were available. These findings indicate that serum NSE may be a useful marker for staging, monitoring treatment, and predicting relapse in patients with small cell lung cancer.

INTRODUCTION

During the past decade, the treatment of small cell carcinoma of the lung has improved considerably such that 50 to 80% of patients with limited-stage disease and 25 to 40% of extensive-stage patients will achieve a complete remission during initial chemotherapy (10). Unfortunately, 75 to 80% of patients with limited-stage disease and nearly all of the patients with extensive-stage disease eventually relapse and die with lung cancer (10, 19). Efforts to improve the dismal prognosis of these individuals have focused not only on attempts to improve chemotherapy and radiation treatment but also on methods of improving or refining current staging techniques (14), monitoring treatment response (29), and predicting relapse (8, 29). A tumor marker which accurately reflects tumor burden and mirrored the clinical condition of individual small cell lung cancer patients could potentially aid in the management of this disease (29).

Recently, large amounts of NSE$^3$ have been detected by both immunoperoxidase staining and radioimmunoassay in a variety of neuroendocrine tumors but in none of the non-neuroendocrine neoplasms tested (27). In order to determine if NSE could serve as a biomarker of small cell lung cancer, we measured the serum NSE levels in 93 newly diagnosed untreated patients treated at our institution between January 1980 and January 1983. The results, reported herein, indicate that serum NSE is a useful biomarker to follow before and during treatment of small cell lung cancer patients and may help predict the recurrence of disease before it is otherwise clinically detected.

MATERIALS AND METHODS

Ninety-three patients with histologically confirmed small cell lung cancer were staged prior to chemotherapy with radionuclide liver and bone scans; radionuclide brain scan, or head computer-assisted tomography scan, or both; fiberoptic bronchoscopy; and bilateral posterior iliac crest bone marrow aspirations and biopsies. Limited-stage disease was defined as tumor confined to the hemithorax and/or ipsilateral mediastinal and suprACLAVICULAR nodules. Patients with disease beyond these sites were classified as extensive disease. All patients received combination chemotherapy with a cyclophosphamide-, doxorubicin-, and vincristine-based regimen administered every 3 to 4 weeks along with whole-brain and thoracic irradiation in selected cases as described previously (9, 11, 13, 30). Prior to each treatment, patients were evaluated with a physical examination, complete blood count, platelet count, and a chest X-ray. Restaging was performed routinely after 6 cycles of chemotherapy and consisted of a repetition of all initially abnormal studies. A complete remission was defined as total clinical and pathological resolution of disease; a partial remission required at least a 50% reduction in the sum of the product of the perpendicular diameters of all measurable lesions or a 50% or greater reduction in all evaluable lesions. Anything less than a partial response was judged as no response. Following completion of chemotherapy, patients were evaluated at 4- to 12-week intervals in the Vanderbilt or Veterans' Hospital oncology clinics with a physical examination, chest roentgenogram, complete blood count, and platelet count. Blood chemistries were obtained when clinically indicated. Any physical or laboratory abnormality suggestive of recurrent disease was evaluated with appropriate confirmatory studies. An effort was made to document all relapses pathologically; however, this was not always possible. In the latter situation, a finding such as the onset of a new neurological finding or a 50% or greater reduction in all evaluable lesions. Anything less than a partial response was judged as no response. Following completion of chemotherapy, patients were evaluated at 4- to 12-week intervals in the Vanderbilt or Veterans' Hospital oncology clinics with a physical examination, chest roentgenogram, complete blood count, and platelet count. Blood chemistries were obtained when clinically indicated. Any physical or laboratory abnormality suggestive of recurrent disease was evaluated with appropriate confirmatory studies. An effort was made to document all relapses pathologically; however, this was not always possible. In the latter situation, a finding such as the onset of a new neurological finding associated with a brain scan abnormality consistent with metastases was taken as clinical evidence of recurrent disease.

Blood was collected from each of the 93 patients at diagnosis, between 9 a.m. and 12 p.m. Serial samples were obtained from 57 of these patients (21 with limited-stage disease; 36 with extensive-stage disease) also between 9 a.m. and 12 p.m., usually at 3- to 6-week intervals during treatment up to the time of restaging but less frequently thereafter. Samples were immediately placed on ice and centrifuged within 30 to 45 min. after which each sample was divided into multiple small aliquots to be stored at $-70^\circ$ until assayed. NSE was prepared from human brain as described previously, and antisera were raised in New Zealand White rabbits (16). The antisera was highly specific as judged by its total lack of reactivity with nonneu-

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3The abbreviations used are: NSE, neuron-specific enolase; NSCLC, non-small cell lung cancer.

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ronal enolase. Serum was assayed in triplicate (50:1 in 0.5 ml total volume) using a double-antibody radioimmunoassay as described previously (20). Intraassay variance was <5% and interassay variance ranged between 5 and 7%.

Student's t test was used to determine the statistical significance between the means.

RESULTS

The mean serum NSE level in 20 healthy adult controls was 9.6 ± 0.7 (S.E.) ng/ml, which is slightly higher than that reported previously (1, 4). A serum NSE greater than 20.0 ng/ml was considered elevated inasmuch as this level is 3 standard deviations above the mean. Overall, 73% (68 to 93) of our patients had an elevated pretreatment serum NSE level. NSE was raised in 23 of 39 (59%) limited-stage patients and 45 of 54 (83%) of patients with extensive-stage disease. The mean pretreatment NSE in limited-stage disease was 33.7 ± 4.7 ng/ml (range, 7.6 to 143.6 ng/ml) versus 94.5 ± 13.8 ng/ml (range, 11.9 to 506.5 ng/ml) for extensive-stage disease (p < 0.001). NSE levels tended to correlate with extent of disease; e.g., the mean NSE level of patients with one site of metastasis is 36.8 ± 4.9 ng/ml (11.2 to 91.2 ng/ml) that for 2 metastatic sites is 96.5 ± 21.0 ng/ml (13.5 to 243.1 ng/ml) (p < 0.001), and that for 3 or more metastatic sites is 186.4 ± 48.2 ng/ml (84.6 to 506.5 ng/ml) (p = 0.05) (Chart 1). All patients with 3 or more sites of metastases had an elevated pretreatment NSE. However, it was not possible to predict specific sites of metastatic disease based on individual NSE levels. Interestingly, patients with isolated central nervous system metastases had elevated pretreatment NSE in only 2 of 6 cases with a mean value lower than that in individuals with other isolated sites of disease (Chart 2).

Serial NSE levels were obtained in 21 patients with limited stage disease, 14 (67%) of whom had an elevated pretreatment value. Of these 21 patients, 20 (95%) achieved a partial or complete remission. Serum NSE declined in 13, remained essentially stable in 4, and increased in 3 (Chart 3). Two of the limited-stage-disease patients with increasing NSE levels at restaging had values which nevertheless remained within the normal range. The single limited-stage-disease patient who failed to respond, i.e., had progressive disease, had a significant increase in the NSE at the time restaging was performed from 7.6 ng/ml at diagnosis to 33.3 ng/ml at restaging. Of the 10 limited-stage-disease patients who achieved a complete remission, the mean NSE at restaging was 17.3 ± 2.2 ng/ml (range, 5.1 to 24.8 ng/ml) versus 21.4 ± 3.4 ng/ml (range, 7.0 to 39.6 ng/ml) for the 10 patients achieving a partial response, a difference not significantly different (p > 0.1).

Relapse data are available for 12 limited-stage-disease patients, 11 (92%) of whom had a significant elevation in serum NSE at the time recurrence was clinically recognized (usually because of an obvious new metastasis or local relapse detected by chest roentgenogram and confirmed by fiberoptic bronchoscopy or biopsy) (Chart 3). The sole patient whose NSE declined at
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Chart 3. A, serial serum NSE levels in small cell lung cancer patients with limited-stage disease at diagnosis, restaging, and relapse; B, serum NSE levels at restaging in limited-stage-disease patients either who remain in remission or for whom serial values are not available. O, NSE levels that are discordant with clinical findings; Q, progressive disease.

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\* PR, partial remission; CR, complete remission.

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relapse recurred with biopsy-proven small cell lung cancer as s.c. nodules. Five limited-stage-disease patients had sufficient serial NSE levels to evaluate the pattern of NSE change prior to clinical recognition of relapse, all of whom had an elevation in NSE from 4 to 12 weeks before clinical recognition of recurrence (mean, 7.2 weeks; Table 1). The course of one such patient is illustrated in Chart 4.

Thirty-two of the 36 (89%) patients with extensive-stage disease and serial measurements of serum NSE had an elevated pretreatment value. The overall response rate of this group of patients was 83% (30 of 36) including 10 complete and 20 partial remissions. Of the responding patients, 27 had had an elevated pretreatment NSE, 26 (96%) of which declined toward normal with treatment (Chart 5). Three patients had clinical responses associated with either a progressive increase or stable serum NSE at restaging. In each of the latter cases, the pretreatment NSE level had been normal or near normal. One responding patient had a pretreatment NSE level that was at the upper limit of normal but which further declined at restaging. Four of 6 (67%) patients with progressive disease had an increase in serum NSE, while 2 (33%) had minimal regression (Chart 5). Nine of 10 (90%)
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Charts. A, NSE determinations in small cell lung cancer patients with extensive-stage disease at diagnosis, restaging, and relapse. O, discordant NSE values. B, NSE levels in extensive-stage-disease patients with progressive disease (○) or at restaging (●) without serial determinations.

Complete responders and 8 of 20 (40%) partial responders had NSE levels <20.0 ng/ml at restaging. The mean NSE value for complete-remission patients was 15.3 ± 1.0 ng/ml (9.9 to 20.8 ng/ml) compared with a mean of 29.1 ± 4.7 ng/ml (7.5 to 94.1 ng/ml) in partial-remission patients (p < 0.01). No extensive-stage-disease patient whose NSE fell to less than 20.0 ng/ml failed to achieve a response.

Relapse data are available for 23 extensive-stage-disease patients, 19 (83%) of whom had clear elevations in serum NSE at the time of clinical relapse (see above; Chart 5). Only one patient had a decline in the NSE at relapse, while 3 patients had stable NSE levels. Two of the latter cases had an elevated pretreatment NSE which had fallen to normal at restaging and remained normal at relapse. One of the patients with stable NSE at relapse had a biopsy-proven solitary central nervous system relapse. The remaining patients relapsed in sites of previous disease including liver, lung, and bone (one biopsy-proven small cell lung cancer; 2 were clinically assessed). A rise in the serum NSE preceded the clinical recognition of relapse in 10 of 18 (56%) extensive-stage-disease patients for whom adequate serial samples were available and did so from 2 to 12 weeks prior to the recognition of recurrent disease (mean, 6.2 weeks; Table 1). Two representative cases are shown in Chart 6.

DISCUSSION

Various attempts to improve the staging and monitoring of therapy of patients with small cell lung cancer by using one or several of the multiple-peptide hormones associated with this cancer have generally met with failure (24). In part, this failure is due to tumor heterogeneity (2). The recent work of Tapi et al. (27) and others (12, 18, 22, 25, 26, 31) demonstrating the presence of NSE in a variety of neuroendocrine tumors but not in non-neuroendocrine cancers suggests that this unique isoenzyme of enolase may be a useful marker for small cell lung cancer. Subsequent studies (1, 4) have shown that serum NSE is elevated at diagnosis in a majority of small cell lung cancer patients prior to treatment. Furthermore, NSE levels tended to correlate with tumor burden, because the highest values were found in patients with metastatic disease while lower values occurred in those with tumor confined to the chest. Importantly, NSE levels declined toward normal in patients demonstrating clinical response to chemotherapy and rose in relapsing patients. Patients with progressive disease generally had increasing NSE at restaging. In both of these previous reports, serial NSE levels were available for only a limited number of patients. As a result, these authors had insufficient data to determine whether changes in serum NSE levels could predict clinical relapse or the development of chemotherapy resistance.

We also found NSE to be elevated at diagnosis in the majority of (73%) of our patients, including 59% of the limited-stage disease patients and 83% of those with extensive disease. NSE levels at diagnosis tended to correlate with the initial tumor burden as evidenced by increasing mean NSE values being associated with increasing number of metastatic sites (Chart 1). An occasional patient with limited-stage disease had a markedly elevated NSE, and a rare patient with extensive-stage disease had a normal value. In both circumstances, errors in staging could account for these discrepancies, or there may have been an occasional error in histological diagnosis. However, since all
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Chart 6. Serial NSE levels in 2 patients with extensive disease; both show a rise in NSE prior to relapse. One patient (top) fails to respond to salvage chemotherapy with concomitant progressive increase of NSE. Salvage therapy has effected a second response in the remaining patient (bottom) with an associated decrease in NSE. CR, complete remission.

pathology was reviewed, the latter appears unlikely. We likewise found a very good correlation between the patients’ clinical courses and subsequent changes in serum NSE, especially in those individuals with elevated pretreatment values. In only 5 patients did the NSE increase at restaging when a response was obtained, while 5 responding patients maintained essentially stable NSE levels. The remaining 40 responding patients all had a decline in NSE at restaging. Interestingly, all of the 10 responding patients (7 limited-stage disease; 3 extensive-stage disease) with a rising or stable NSE level at restaging had had a normal or nearly normal pretreatment NSE.

Only 2 patients (one limited-stage disease; one extensive-stage disease) had a fall in serum NSE at relapse, although 3 patients (all extensive-stage disease) had stable NSE levels; the remaining 30 relapsing patients had a definite rise in serum NSE upon tumor recurrence. A stable or declining NSE in relapsing patients may have occurred for a number of reasons including the growth of tumor cells devoid of NSE, tumor cells containing NSE may have failed to release it into serum, or there may have been a change in histology at relapse to a non-small cell type (3, 5). The latter seems unlikely in our series since patients who were rebiopsied at relapse had small cell lung cancer histologically. Thus, from our data, we can only conclude that NSE does not always increase at relapse, for reasons which are unknown.

Of particular interest are the 23 patients who had multiple NSE values obtained before the clinical recognition of relapse. Fifteen (65%) of these patients had a clear elevation in NSE that predated the clinical recognition of relapse by as many as 12 weeks. Importantly, every patient who achieved a partial or complete response accompanied by a fall in NSE and whose NSE level subsequently increased following restaging and cessation of chemotherapy eventually relapsed; i.e., there were no “false-positive” increases in NSE levels.

Four limited-stage-disease patients remain in clinical complete remission; 2 have stable and normal NSE levels at 7+ and 28+ months from diagnosis. The other 2 patients, who are at 12+ and 20+ months from diagnosis, have had recent slight increases in serum NSE values but thus far have been without evidence of relapse for 8 and 4 weeks, respectively. Clearly, if NSE is to be useful in the management of small cell lung cancer, this predictive property is important and requires verification. The predictive property of NSE resembles the behavior of carcinoembryonic antigen (29) and neurophysins (17), both of which appear to increase in relapsing small cell lung cancer patients as many as 3 months before the clinical recognition of recurrent disease. However, these and other putative markers of small cell lung cancer have been associated with a heterogeneity of expression, while NSE has thus far been found in all cell lines of small cell lung cancer tested (15).

NSE has not yet been fully evaluated in patients with NSCLC although Marangos et al. (15) have reported a clear distinction in the NSE levels measured in tissue cultures of small cell lung cancer as compared with NSCLC. However, as with other enzymes originally thought to be found exclusively in neuroendocrine cells (6), NSE has been detected recently in a few patients with NSCLC. Ariyoshi et al. (1) reported elevated NSE levels in 11% of 54 patients with NSCLC. Perhaps of note, all of these patients had either large cell lung cancer exclusively or large cell mixed with other non-small cell histologies. This is particularly noteworthy in light of the findings of Gazdar et al. (6, 7), who reported in vitro “conversion” of small cell lung cancer to large cell cancer morphologically. Although “transformed” cells had lost most or all of their “amine precursor uptake and decarboxylation characteristics,” certain neuroendocrine features such as creatine kinase-BB isoenzyme activity were retained. NSE was not evaluated. Thus, it is possible that the large cell lung cancers described by Ariyoshi were originally small cell tumors that “changed” histology (5) but “retained” NSE activity or were mixed tumors (23) with the small cell component incompletely eradicated by therapy. Further work is necessary to clarify this issue.

In summary, NSE is elevated at diagnosis in a majority of patients with small cell lung cancer, especially those with extensive stage disease. NSE levels correlate with tumor burden and generally reflect the clinical course of disease failing with remission and rising with relapse. Our data suggest that, although the return of NSE to normal is unfortunately not an assurance of long-term survival, its sustained normalization appears to be a prerequisite for cure. Of equal importance is the observation that, in two-thirds of the patients evaluated, an increase in serum NSE actually predated the clinical recognition of relapsed disease. This latter property, if it proves reproducible, may be useful in the future management of patients with small cell lung cancer in that it would allow for the early institution of different therapy at a time when it is likely to be most beneficial, i.e., when the tumor burden is low. While at present salvage regimens for relapsed small cell lung cancer are lacking in sustained efficacy, the apparent synergism of cisplatin and etoposide hold promise for more effective treatment regimens in the future (28). Serum NSE and immunoperoxidase staining also may eventually prove useful in distinguishing small cell lung cancer from non-small cell lung cancer in patients whose tumors are particularly anaplastic.
defying light microscopic characterization. Further prospective studies are under way to fully define the place of this and other potential biomarkers (7, 17, 21, 29) in the management of small cell lung cancer.

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