Significance of Neuron-specific Enolase Levels before and during Therapy for Small Cell Lung Cancer

James A. Bonner, Jeff A. Sloan, Kendrith M. Rowland, Jr., George G. Klee, John W. Kugler, James A. Mailliard, Martin Wiesenfeld, James E. Krook, Andrew W. Maksymiuk, Edward G. Shaw, Randolph S. Marks, and Edith A. Perez

Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905 [J. A. B., J. A. S., G. G. K., E. G. S., R. S. M.]; Carle Cancer Center CCOP, Urbana, Illinois 61801 [K. M. R.]; Illinois Oncology Research Association CCOP, Peoria, Illinois 61602 [J. W. K.]; Nebraska Oncology Group, Creighton University, University of Nebraska Medical Center and Associates, Omaha, Nebraska 68131 [J. A. M.]; Cedar Rapids Oncology Project CCOP, Cedar Rapids, Iowa 52403 [M. W. J.]; Duluth CCOP, Duluth, Minnesota 55805 [J. E. K.]; Saskatoon Cancer Centre, Saskatoon, Saskatchewan, S7N 4H4 Canada [A. W. M.]; and Mayo Clinic Jacksonville, Jacksonville, Florida 32224 [E. A. P.]

ABSTRACT
The level of serum neuron-specific enolase (NSE) has been implicated as a prognostic factor for patients with small cell lung cancer (SCLC). A prospective evaluation was undertaken to assess the prognostic significance of pretreatment NSE and treatment-induced minimum NSE values in patients with SCLC. Patients from two Phase III North Central Cancer Treatment Group trials [one for patients with extensive stage SCLC and one for patients with limited stage SCLC] were asked to enter this laboratory correlational trial. Both trials included treatment with four to six cycles of etoposide and cisplatin, and 121 patients (71 extensive stage SCLC and 50 limited stage SCLC) were entered into the present study of NSE. Pretreatment NSE values and treatment-induced minimum NSE values were independent predictors of time to progression and survival in multivariate analysis. Hazard rate modeling allowed the formulation of specific relationships of NSE to time to progression and survival. Pretreatment NSE levels inversely correlated with time to progression and survival in these patients with SCLC. Pretreatment NSE accounted for 28% of the variance in survival. Both pretreatment NSE and treatment-induced minimum NSE were independent prognostic predictors of time to progression and survival.

INTRODUCTION
The enolase enzymes are involved in the glycolysis pathway at the conversion of 2-phosphoglycerate to phosphoenolpyruvate (1, 2). Glycolysis is the process of anaerobic degradation of glucose to lactic acid, but it is also an important preparatory pathway for aerobic glucose catabolism. There are slight structural variations in the enolases that predominate in various organ systems, and these variations appear to be related to the environmental conditions that may be present in various organ systems. For instance, NSE is the predominant enolase found in neural tissue, and the structural characteristics of this enolase allow for greater stability in high chloride concentrations compared with the enolases of other organ systems. This structural characteristic is thought to be important for enolase function in neural tissue because depolarizations result in transient high concentrations of chloride.

With the development of antiserum to the enolase enzymes, it became apparent that patients with the diagnosis of SCLC frequently had increased levels of NSE compared with control patients (2–15). Subsequently, it was discovered that NSE levels correlated with the patients’ extent of disease (2, 7, 9, 13, 16), and therefore, the question arose as to whether NSE might be a tumor marker that would allow the clinician an improved capacity to assess an individual patient’s prognosis and additionally whether NSE could be used to assess the potential for an individual patient to respond to various therapies.

Therefore, the North Central Cancer Treatment Group embarked on a prospective study of NSE in patients with SCLC with the following goals: (a) determine whether baseline NSE levels correlated with prognosis or response to therapy (or both); and (b) determine whether the response of NSE after therapy correlated with tumor response or patient survival (or both). To investigate these two goals, patients from two prospective studies, one for patients with SSSCLC and one for patients with LSSCLC, were asked to participate in a prospective assessment of NSE levels before and during treatment of their SCLC.

PATIENTS AND METHODS
A prospective laboratory correlational study was undertaken to determine the significance of pretreatment, and the treatment-induced minimum NSE values for patients with SCLC (both extensive stage disease and limited stage disease).

3 The abbreviations used are: NSE, neuron-specific enolase; SSSCLC, extensive stage small cell lung cancer; LSSCLC, limited stage small cell lung cancer; SCLC, small cell lung cancer.
Patients from two North Central Cancer Treatment Group prospective randomized trials were allowed to enter onto this laboratory correlational study. The eligibility for patients in the first trial included only those with ESSCLC, and the eligibility for patients in the second trial included only those with LSSCLC.

These trials were selected because both trials included primary treatment with combination chemotherapy consisting of etoposide and cisplatin. The patients who were entered on the trial for ESSCLC (August 1990-July 1993) were treated with 4 cycles of etoposide (100 mg/m²) and cisplatin (30 mg/m²) on days 1, 2, and 3 of each monthly cycle. They also were randomized to treatment with daily megestrol for 2 years (beginning days 3–5) or placebo. The details of treatment and the results of this trial have been published (17). The use of megestrol did not significantly affect time to progression or survival compared with placebo (17). The patients who were entered on the trial for LSSCLC (September 1990–November 1996) were treated with six cycles of etoposide and cisplatin. The dose schedules of etoposide and cisplatin were identical to the first trial for cycles four to six (whereas the patients received thoracic radiation therapy during cycles four and five); however, the trial for cycles four to six (whereas the patients received thoracic radiation therapy during cycles four and five) had a different dose schedule. They also were randomized to treatment with daily megestrol for 2 years (beginning days 3–5) or placebo. The details of treatment and the results of this trial have been published (17). The use of megestrol did not significantly affect time to progression or survival compared with placebo (17). The patients who were entered on the trial for LSSCLC (September 1990–November 1996) were treated with six cycles of etoposide and cisplatin. The dose schedules of etoposide and cisplatin were identical to the first trial for cycles four to six (whereas the patients received thoracic radiation therapy during cycles four and five); however, the etoposide dose was slightly higher (than the trial for the patients with ESSCLC) for the first three cycles: 130 mg/m². This trial included a randomization to once-daily thoracic radiation therapy (50.4 Gy in 28 fractions) or twice-daily thoracic radiation therapy (48 Gy:1.5 Gy twice daily with a 2-week break after 24 Gy; Ref. 18).

Of the 567 patients (243 on the ESSCLC trial and 324 on the LSSCLC trial) who were entered into the two trials, 121 participated in the laboratory correlational study involving sampling of NSE (71 with ESSCLC and 50 with LSSCLC) before and during treatment (April 1991–November 1992). All patients signed an Institutional Review Board-approved statement of informed consent. Of the 19 institutions that participated in the ESSCLC and LSSCLC trials, 18 and 16 participated in this trial, respectively. Patients who were entered on this trial were assessed for NSE at the pretreatment visit, each visit during treatment, and each follow-up visit (every 4 months for 3 years, followed by every 6 months) until the time of progression. The assay for NSE was an immunochemiluminescence assay that used two monoclonal antibodies and was developed in an agreement with Hybritech and Dr. George Klee of the Mayo Foundation (19).

The significance of the pretreatment NSE and minimum NSE was assessed by hazard rate modeling in an effort to determine whether a mathematical relationship existed between these values and time to progression and overall survival (from study entry; Refs. 20 and 21). Additionally, Spearman correlation coefficients were calculated for the above potential relationships (22). A multivariate analysis by the method of Cox was used to determine independent prognostic factors (23). Mean values were expressed with associated 95% confidence intervals.

Survival curves were constructed by the method of Kaplan and Meier (24), and comparisons of these curves were made by the log-rank test (25).

RESULTS

Of the 121 patients who were entered into this laboratory correlational study, the median age was 63 years (range, 30 to 74 years). The median follow-up time for the living patients was 3.2 years, with a median potential follow-up time of 15 months. Of the 121 patients, 105 died. The initial goal of this study was to determine the significance of the pretreatment NSE level. It was determined that there was a statistically significant difference in the pretreatment NSE levels of patients who had ESSCLC compared with those who had LSSCLC, with mean values of 254 ± 36.4 ng/ml and 47.1 ± 4.9 ng/ml, respectively (P < 0.0001). A group of 100 normal adult volunteers showed a mean value of 16.4 ± 0.4 ng/ml. This mean value for the group of normal adults is somewhat higher than those of other published series in which means have generally been ~12 ng/ml; however, it is certainly consistent with slight variability in various control populations (2–15).

The above results suggested that the baseline NSE value was of prognostic significance regarding the extent of disease (i.e., extensive disease versus limited disease). Of the 121 patients who were entered into the study, 53 had a best response of complete response. Patients who achieved this complete response during therapy or immediately after therapy had a pretreatment median NSE value of 53 ng/ml compared with a median value of 133 ng/ml for patients who achieved less than a complete response (P = 0.01). A scatterplot of baseline NSE versus time to progression was constructed to initially assess the possibility of an inverse relationship (Fig. 1, left). Initial visual inspection of the scatterplot revealed a suggestion that the patients with higher baseline NSE values were more likely to have shorter periods from registration to time of progression.

Hazard modeling for progression rate based on NSE was performed, and the following equation describing the relationship of NSE and time to progression was determined: H.R.(t) = (Baseline H.R. at time t) × e^{0.0024 (NSE value)}, where H.R. is the hazard rate.

An example of the usefulness of this modeling is as follows. A 30-unit increase in baseline NSE would result in a 7.4% increase in the hazard rate of disease progression at any particular time. Because an inverse relationship between baseline NSE and time to progression was found, a scatterplot of baseline NSE versus overall survival was created to initially assess whether a visual relationship existed between these variables (Fig. 1, right). The scatterplot suggested a possible relationship, and mathematical modeling of the hazard rate of death with respect to NSE was performed. The following equation describing the relationship of NSE to overall survival was determined: H.R.(t) = (Baseline H.R. at time t) × e^{0.0017 (NSE value)}, where H.R. is the hazard rate.

Although the equation describing the relationship between the hazard rate of death and pretreatment NSE resulted in a slightly reduced coefficient of NSE compared with the coefficient for the relationship of NSE to time to progression, there remained a statistically significant relationship between NSE and the hazard rate of death (P < 0.05). This relationship between pretreatment NSE and the hazard rate of death was more easily illustrated by calculating a Spearman correlation coefficient of the relationship (r = −0.53, P = 0.0001). The
square of this correlation coefficient was used to derive the fact that 28% of the variance in survival was attributed to NSE, and this percentage is a high level of variance attributable to a single biological factor.

Next, a multivariate analysis was performed to assess whether pretreatment NSE was an independent prognostic factor. The following potential prognostic factors were included in the analysis: pretreatment NSE, treatment-induced minimum NSE during the treatment course, age, sex, stage of disease (extensive or limited), and performance status (0 versus 1, versus 2). Pretreatment NSE and minimum NSE were independent prognostic indicators for time to progression and survival (Table 1).

Because the treatment-induced minimum NSE level was an independent treatment-associated measure of time to progression and overall survival, further analyses of this end point were undertaken. The median number of NSE samples/patient was 5, with a range of 2–13. The median time between the baseline NSE and the final NSE sample was 161 days, with a range of 16–928 days. The median time to the minimum NSE value was 91 days. The median minimum value was used to separate patients into two prognostic groups. This cutoff value was 12 ng/ml for the overall population of patients. Patients with limited or extensive stage disease who achieved minimum NSE values of less than the cutoff value had statistically significant improvements in time to progression and overall survival (Figs. 2 and 3).

**DISCUSSION**

This prospective study of patients with LSSCLC and ESSCLC revealed that the pretreatment serum NSE level was inversely correlated with survival, and in the present study, this one prognostic variable (NSE) appeared to account for 28% of the variance in survival. Also, the pretreatment NSE level was inversely correlated with response to treatment and the time to progression. Unlike many trials, this trial evaluated NSE levels prospectively during treatment, and therefore an assessment of the patients’ treatment-induced minimum NSE (during treatment) could be made. In the Cox regression model, both the pretreatment NSE and the minimum NSE (as well as stage) were of independent prognostic significance with respect to survival.

Other authors have examined the prognostic significance of serum NSE in patients with SCLC (7, 12, 13, 16, 26). In a recent study by Jorgensen et al. (13), 770 patients were studied from nine institutions. They found that pretreatment NSE was the most powerful predictor of survival, followed by performance status and stage of disease. They developed a prognostic index evaluation system that assigned each patient an overall score based on the sum of scores given for performance status, stage, and NSE. This system proved to be fairly useful in categorizing four distinct prognostic groups. The strong dependence of this system on pretreatment NSE corroborates the results of the present series. They did not evaluate the prognostic significance of treatment-induced minimum NSE.

Others also have confirmed the prognostic significance of pretreatment serum NSE values, but only a few reports of small numbers of patients have examined serial serum NSE levels that have allowed for the assessment of treatment-induced minimum NSE levels. Nou et al. (16) performed a longitudinal study of NSE levels in patients with SCLC. They acquired pretreatment NSE levels and NSE levels at the time of response in 62 patients. In the group of 39 patients with limited-stage disease, the mean survival was 26.4 months if the NSE at response was <12 ng/ml, and this survival appeared to compare favorably with the overall survival of the patients with limited-stage

**Fig. 1** The scatterplots suggest an inverse relationship between pretreatment NSE and time to progression (left) or survival (right).

<table>
<thead>
<tr>
<th>Factor</th>
<th>( P ) (progression) ( P ) (survival)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td>0.0001</td>
</tr>
<tr>
<td>PS( ^b )</td>
<td>0.65</td>
</tr>
<tr>
<td>Age</td>
<td>0.36</td>
</tr>
<tr>
<td>Sex</td>
<td>0.18</td>
</tr>
<tr>
<td>Baseline NSE</td>
<td>0.0001</td>
</tr>
<tr>
<td>Treatment-induced minimum NSE</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

\(^a\) Time to progression.

\(^b\) PS, performance status.
disease (although statistical comparisons were not done). The results of the series by Nou et al. (16) support the concept that certain cutoff values may be of prognostic significance as minimum values during treatment. As in the present series, the value of 12 ng/ml appeared to be an important cutoff value for the minimum NSE level. Additional support for this concept comes from a study reported by Johnson et al. (26). An inverse relationship existed for the treatment-induced minimum NSE and time to progression (26), similar to the results in the present study (Fig. 2).

The biological ramifications of the clinical associations between NSE and the time to progression or overall survival of patients with SCLC are beginning to be explored (27, 28). Recently, Brodin et al. (27) described a change in radiosensitivity over a 6-month period of a SCLC line (U1906) grown in culture. During the 6 months of culture, the cell line became radioresistant compared with its initial sensitivity. The radioresistant change was associated with an increased capacity to repair radiation-induced damage, as illustrated by an increased shoulder on the radiation survival curve. Also, the radioresistant change was associated with increased adherence, decreased cytokeratin content, and increased glucagon and NSE production. After 6 months of culture, the cells' morphology was more neural in appearance (i.e., pseudopodia developed). The results from Brodin et al. (27) raise the question of whether a mechanistic link may exist between NSE production and response to current treatments. The general concept regarding the degree of neural differentiation and its relationship to therapeutic response is the subject of ongoing in vitro (13) and clinical studies. Further work is necessary to completely decipher the role of NSE in the response to therapy and determine whether the potential exists for the manipulation of response through the manipulation of NSE.

ACKNOWLEDGMENTS

We thank the following groups and persons for their participation: Geisinger Clinic and Medical Center CCOP, Danville, PA 17822 (Dr. Suresh Nair); Rapid City Regional Oncology Group, Rapid City, SD
REFERENCES


Significance of Neuron-specific Enolase Levels before and during Therapy for Small Cell Lung Cancer

James A. Bonner, Jeff A. Sloan, Kendrith M. Rowland, Jr., et al.


**Updated version**
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/6/2/597

**Cited articles**
This article cites 25 articles, 2 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/6/2/597.full#ref-list-1

**Citing articles**
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/6/2/597.full#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.