Serum Levels of Pro-Gastrin-releasing Peptide for Follow-Up of Patients with Small Cell Lung Cancer

Takiji Okusaka,1 Kenji Eguchi, Takashi Kasai, Takayasu Kurata, Nobuyuki Yamamoto, Yuichiro Ohe, Tomohide Tamura, Tetsu Shinkai, and Nagahiro Saijo

Department of Internal Medicine, National Cancer Center Hospital [T. O., K. E., H. K., T. K., T. K., N. Y., Y. O., T. T., T. S.], and Pharmacology Division, National Cancer Center Research Institute [N. S.], 5-1-1 Tsukiji, Chu-ku, Tokyo 104, Japan

ABSTRACT
To assess the clinical usefulness of serum pro-gastrin-releasing peptide (Pro-GRP) as a tumor marker for small cell lung carcinoma (SCLC), we measured serum levels of Pro-GRP with a newly developed ELISA and measured serum levels of neuron-specific enolase (NSE) in 44 patients with untreated SCLC and 77 patients with untreated non-SCLC. We prospectively measured serum levels of Pro-GRP and NSE in SCLC patients after initial treatment until relapse. The sensitivity (70%) and specificity (91%) of Pro-GRP were similar to those of NSE (70 and 86%). Thirty-nine % of patients who had a partial response still had elevated serum levels of Pro-GRP at the time of restaging after initial treatment. In follow-up study, 94% of patients had elevated serum levels of Pro-GRP again at the time of relapse, whereas 37% of patients showed elevated levels of NSE. Levels of Pro-GRP increased a median of 35 (−95 to 151) days before clinical evidence of relapse was detected with successive physical examinations and imaging studies, whereas levels of NSE increased 20 (−85 to 124) days after relapse was detected (P < 0.05). Pro-GRP was helpful as a diagnostic aid and a marker for therapeutic effect and relapse in patients with SCLC, supplemented to serum NSE.

INTRODUCTION
Although SCLC2 is highly sensitive to systemic chemotherapy, the prognosis of patients is extremely poor. Because SCLC often recurs and becomes resistant to treatment, early diagnosis, more effective treatment, more accurate evaluation of treatment, and early detection of relapse are needed to improve survival of patients with SCLC. GRP is a gut peptide hormone originally isolated from porcine stomach. It can increase plasma levels of gastrin in dogs (1), and GRP is present in nerve fibers in non-antral stomach tissue, brain, and neuroendocrine cells in fetal lung (2–4). Because it is often produced by SCLC cells (5, 6), GRP might be a useful tumor marker in patients with SCLC (7). However, GRP is unstable in blood and is difficult to measure in clinical situations. On the other hand, Pro-GRP (31–98), a region common to three types of human Pro-GRP, is stable in blood and can be measured by RIA (8). The recent development of a more convenient ELISA might allow Pro-GRP to be serve as a tumor marker for SCLC, which is more sensitive, specific, and reliable than NSE (9, 10). To determine whether Pro-GRP is useful for diagnosis, monitoring of treatment effects, early recognition of relapse, and determining prognosis, we measured serum levels of Pro-GRP and NSE in patients with SCLC before and after initial treatment.

PATIENTS AND METHODS
Untreated patients with SCLC that had been proven pathologically or cytologically or both were eligible for this study. Clinical staging was based on results of clinical and laboratory examinations, chest radiography, computed tomography (chest and brain), abdominal ultrasonography, bone scintigraphy, and bone marrow aspiration. The Veterans’ Administration Lung Study Group criteria for LD and ED were applied (11). Patients with ED received six cycles of chemotherapy with PVP, alternating chemotherapy with CAV (cyclophosphamide, doxorubicin, and vincristine) and PVP, or dose-intensive weekly chemotherapy with CODE (cisplatin, vincristine, doxorubicin, and etoposide). Patients with LD were treated with four cycles of chemotherapy with PVP alone or PVP and sequential or concurrent thoracic irradiation. Patients were monitored with measurements of serum levels of Pro-GRP and NSE every 2 weeks during treatment and every month after restaging. Responses to therapy were evaluated with imaging studies, and the overall response to treatment was analyzed according to WHO criteria (12). To detect relapse, patients were examined regularly as follows: physical examinations and chest X-ray every month; abdominal ultrasonography and brain and chest computed tomography every 3 months; and bone scintigraphy every 5 months. Serum levels of Pro-GRP were measured with ELISA (TND-4 kit; Terumo Co., Kanagawa, Japan, and Tonen Co., Saitama, Japan). Serum levels of NSE were measured by enzyme-immunoassay kit purchased from Eiken Chemicals (Tokyo, Japan). The same serum samples were used to measure levels of both Pro-GRP and NSE.

We analyzed the sensitivity, specificity, efficiency, and positive and negative predictive values of the two tumor markers. Serum levels of Pro-GRP and NSE were considered to be elevated when they exceeded 46 pg/ml and 6.4 ng/ml, respectively, which are the mean + 3 SD of value in healthy subjects. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1To whom requests for reprints should be addressed. Phone: 81-3-3542-2511; Fax: 81-3-3545-3567.
2The abbreviations used are: SCLC, small cell lung cancer; NSCLC, non-SCLC; GRP, gastrin-releasing peptide; NSE, neuron-specific enolase; LD, limited disease; ED, extensive disease; PVP, cisplatin and etoposide; CR, complete response; PR, partial response.
estimated with logarithmic transformation by Kodama et al. (13). We defined the day of increase of Pro-GRP or NSE as the day when the values of the tumor marker changed in at least one of the following ways: (a) the serum level increased by more than 10% of the previous concentration on 2 consecutive days; or (b) increased by more than 50% of the previous concentration. For definition of these criteria, we referred to intraassay and between-day coefficient of variations of the assay kits (Pro-GRP: 1.7-4.6% and 4.2-6.8%, respectively; NSE: 2.2-3.6% and 4.4-5.6%, respectively; Ref. 9). We defined the day of relapse as the day when relapse was detected with imaging studies or physical examination. The lead time was defined as the period from the day of increase to the day of relapse. This study was approved by the Institutional Review Board of the National Cancer Center, Tokyo. Informed consent was obtained from all patients.

Mann-Whitney U test was used to determine the significant differences in median serum levels of Pro-GRP and NSE between patients with NSCLC and with SCLC and between LD and ED patients and in median lead times between serum levels of Pro-GRP and NSE. The increment ratios (serum concentration divided by the cutoff value) between serum levels of Pro-GRP and NSE were compared using Wilcoxon signed-ranks test. The $\chi^2$ test was used to examine the differences in the rates of patients who had elevated levels of Pro-GRP and NSE. All $P$s were two-tailed, and differences with $P$s less than 0.05 were considered significant. Survival curves were calculated with the Kaplan-Meier method (14), and differences between survival curves were evaluated with the log-rank test (15, 16).

RESULTS

Pro-GRP and NSE Levels in SCLC Patients and NSCLC Patients Before Treatment. Between November 1993 and April 1995, 44 untreated patients with SCLC and 77 patients with NSCLC were entered into this study at the National Cancer Center Hospital in Tokyo (Table 1). The median follow-up time of patients with SCLC was 11.4 months. Twenty patients with SCLC were classified as having LD, and 24 patients with SCLC as having ED.

The sensitivity and specificity of Pro-GRP were similar to those of NSE (70% versus 70% and 91% versus 86%, respectively; Table 2). In the 77 patients with NSCLC, the median serum levels of Pro-GRP and NSE were 27.6 (12.4-68.8) ng/ml and 3.7 (1.6-86.6) ng/ml, respectively. In the 44 patients with SCLC, the median serum levels of Pro-GRP and NSE were 226.7 (7.2-6786.0) pg/ml and 10.1 (2.0-530.0) ng/ml, respectively. There were significant differences in the levels of Pro-GRP and NSE between the patients with NSCLC and with SCLC ($P < 0.01$). In patients with SCLC, the increment ratio (serum concentration divided by the cutoff value) for Pro-GRP was significantly higher than that for NSE [median: 4.9 (0.2-147.5) versus 1.6 (0.3-82.8); $P < 0.01$].

Levels of Pro-GRP and NSE in untreated patients with SCLC were poorly correlated ($r = 0.103$; Fig. 1). Forty of 44 SCLC patients (91%) had elevated serum levels of Pro-GRP or NSE or both. In 43% of patients with SCLC (19 of 44 patients), only one of the tumor markers was elevated: NSE alone in 10 patients (23%) or Pro-GRP alone in 9 patients (20%).

In the 20 patients with LD, the median serum levels of Pro-GRP and NSE were 51.2 (7.2-2596.0) pg/ml and 6.5 (2.9-75.7) ng/ml, respectively, and the increment ratio for Pro-GRP tended to be higher than that for NSE [median: 1.1 (0.2-56.4) versus 1.0 (0.5-11.8); $P < 0.052$]. In the 24 patients with ED,

### Table 1 Patient characteristics

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<thead>
<tr>
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<th>SCLC</th>
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### Table 2 Evaluation of usefulness of serum levels of Pro-GRP and NSE for diagnosis of SCLC

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<th>Pro-GRP</th>
<th>NSE</th>
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[^a]: Analyzed in 44 patients with SCLC and 77 patients with NSCLC.  
[^b]: If either Pro-GRP or NSE were positive, the overall result was positive.

Fig. 1 Serum concentrations of Pro-GRP and NSE in 44 patients with SCLC. The correlation coefficient was 0.103.

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the median serum levels of Pro-GRP and NSE were 399.6 (16.1–6786.0) pg/ml and 20.5 (2.0–530.0) ng/ml, respectively. In ED patients, the increment ratio for Pro-GRP was significantly higher than that for NSE [median: 8.7 (0.4–147.5) versus 3.2 (0.3–82.8); \( P < 0.011 \)]. However, in both LD and ED patients, there were no significant differences in the rates of patients who had elevated levels of Pro-GRP and NSE (LD: 60% versus 55% and ED: 79% versus 83%). There was significant difference in the levels of NSE between the patients with LD and with ED (\( P < 0.01 \)).

### Changes in Serum Pro-GRP and NSE Levels following Treatment.
Among patients who had elevated levels of Pro-GRP before treatment, 2 patients who had CR and 7 patients who had PR still had elevated levels at restaging (2 of 8 patients, 25%, and 7 of 18 patients, 39%, respectively). On the other hand, serum levels of NSE at restaging were normal in all patients who achieved CR and PR. Of the six patients who showed no change or progressive disease, none showed definite changes in their serum levels of Pro-GRP and NSE after treatment (Fig. 2).

### Change in Serum Pro-GRP and NSE Levels until Relapse.
Twenty-six patients had relapsed by the time of final analysis. The rate of patients who had elevated Pro-GRP levels at the time of relapse among patients who had elevated Pro-GRP levels before treatment was significantly higher than the rate of patients who had elevated NSE levels at the time of relapse among patients who had elevated NSE levels before treatment (17 of 18 patients, 94%, versus 7 of 19 patients, 37%, respectively; \( P < 0.01 \)).

### Time Lag between the Day of Relapse and the Day of Increase in Serum Pro-GRP and NSE on Prospective Observation.
At the time of final analysis, 18 of 36 patients who had achieved CR or PR with initial treatment had relapsed. Of these 18 patients, the rate of patients in whom serum levels of Pro-GRP had increased before clinically proven relapse (see “Patients and Methods”) was significantly higher than that of patients in whom serum levels of NSE increased earlier than clinically proven relapse (16 patients, 89%, versus 7 patients, 39%; \( P < 0.01 \)). Levels of Pro-GRP increased a median of 35 (−95 to 151) days before the day of relapse, whereas levels of NSE increased a median of 20 (−85 to 124) days later than the day of relapse (\( P < 0.05 \); Fig. 3).

### Survival and Change in Serum Pro-GRP and NSE Levels in Patients Who Achieved PR.
Twenty-three of the 44 SCLC patients had died of cancer by the time of final analysis. We calculated survival in 11 of 18 PR patients whose levels of Pro-GRP had decreased to the normal range by the time of restaging (group A; 4 patients with LD and 7 patients with ED) and 7 whose levels of Pro-GRP remained elevated (group B; 2 patients with LD and 5 patients with ED). The survival period of group A was significantly longer than that of group B (\( P < 0.05 \) by log-rank test; Fig. 4).

### DISCUSSION
In 1994, Miyake et al. (8) developed a RIA system to measure serum Pro-GRP and reported that Pro-GRP was a tumor marker specific for SCLC. As a more convenient assay
Pro-GRP and NSE. There was a significant difference in lead time between Pro-GRP and NSE [median: 35 (−95 to 151) days and −20 (−124 to 85) days, respectively; \( P < 0.05 \) by Mann-Whitney U test]. Day 0 shows the day of relapse. + and −, earlier and later than the day of relapse, respectively.

In present study, we used ELISA to measure serum levels of Pro-GRP prospectively to evaluate the clinical usefulness of Pro-GRP. We investigated the correlation between changes in levels of Pro-GRP and the interval between increases in serum levels and relapse. The sensitivity and specificity of measurement of serum levels of Pro-GRP were similar to those of NSE. The two tumor markers have been suggested to have similar efficacy for differentiating SCLC from NSCLC. However, the increment ratios, which indicate ranges of distribution of values for the serum levels of Pro-GRP, were significantly higher than those for NSE. Measurements of both Pro-GRP and NSE may be clinically useful because the correlation coefficient between these two tumor markers was low (\( r = 0.103 \)), and some patients with SCLC were positive for only NSE (10 of 44, 23%) or Pro-GRP (9 of 44, 20%). If the overall result was considered positive when at least one of these tumor markers was positive, the sensitivity (91%) and specificity (78%) was high.

Changes in serum levels of Pro-GRP after treatment were useful for monitoring therapeutic effect. Changes in serum levels of Pro-GRP were particularly useful in patients who had achieved PR, because levels at restaging were not always within the normal range, although serum levels of NSE in all PR patients were below the cutoff value. Additionally, PR patients in whom levels of Pro-GRP were below the cutoff value at restaging survived significantly longer than did PR patients in whom levels of Pro-GRP remained elevated. For indicating viable residual tumor after treatment, sustained levels of Pro-GRP were more reliable than sustained levels of NSE.

The present study also demonstrated that measurement of serum levels of Pro-GRP was useful for the early recognition of relapse in patients with SCLC. Seventeen of 18 (94%) patients who had elevated levels of Pro-GRP before treatment had elevated levels of Pro-GRP again at relapse. Because its lead time was longer than that of NSE, Pro-GRP will be more helpful for predicting relapse. Re-elevation of serum levels of Pro-GRP in treated patients with SCLC may allow an earlier retreatment with different modalities.

In conclusion, the present study showed that Pro-GRP was
helpful for diagnosis, monitoring therapeutic effects, and early recognition of relapse, supplemented to serum NSE. However Pro-GRP was not a highly specific tumor marker such as β-humanchorionic gonadotropin for decision making of treatment in patients with germ cell tumor.

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