The Diagnostic and Prognostic Value of ProGRP in Lung Cancer

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Abstract. *Aim: To investigate the diagnostic and prognostic significance of pro-gastrin-releasing peptide (ProGRP) in non-small cell (NSCLC) and small cell lung cancer (SCLC) and compare this marker with other known serum markers in lung cancer. Patients and Methods: Serum levels of ProGRP, neuron-specific enolase (NSE), CYFRA 21-1 and carcinoembryonic antigen (CEA) were measured in 37 patients with benign pulmonary disease (BPD), 88 with advanced NSCLC and 37 with SCLC. Results: The ProGRP assay showed a better clinical performance than that of NSE in discriminating between SCLC and BPD or NSCLC, especially at specificity higher than 90%. ProGRP and NSE sensitivity in SCLC at 95% specificity versus the BPD group was 78.4% and 48.6%, (p=0.001) and at 97.7% specificity versus NSCLC, 75.7% and 37.8%, respectively (p=0.001). A significant association of low ProGRP levels with high-grade NSCLC tumors was found (p=0.002). A univariate analysis showed a significant association of ProGRP with survival both in NSCLC and SCLC (p=0.03 and p=0.04, respectively). In multivariate analysis, performance status (PS) and CYFRA 21-1 in NSCLC, and PS, CYFRA 21-1 and serum lactic dehydrogenase in SCLC were found as significant variables with an independent impact on survival. Conclusion: ProGRP is a useful marker in SCLC, with diagnostic performance better than that of NSE and demonstrating association with survival in NSCLC and SCLC limited to univariate analysis.*

Lung cancer is the most frequent and the most deadly malignancy, resulting in an enormous global health problem. The majority of lung cancer patients present with inoperable, advanced, disease entailing a poor prognosis.

Lung cancer is classified into two major entities depending on cell type: small cell lung cancer and non-small cell lung cancer (SCLC and NSCLC, respectively). SCLC accounts for up to 15% of all new lung cancer cases and differs biologically from NSCLC by the presence of neuroendocrine differentiation and a higher rate of tumor growth. Clinically, early metastatic spread is very common. SCLC is highly sensitive to initial chemotherapy and radiotherapy (1); however, relapse occurs in most patients, particularly those with extensive disease and acquired resistance to second-line therapy is evident.

In an attempt to improve the management of lung cancer patients, methods to detect substances released by tumor cells into the circulation, known as serum tumor markers, have been developed. A panel of tumor markers has been investigated for their value in lung cancer (2). Carcinoembryonic antigen (CEA) and CYFRA 21-1 are now considered to be the markers of choice in lung cancer, however, these markers are not specific for lung cancer. Furthermore, high levels of these markers can be found both in NSCLC and SCLC. Although neuron-specific enolase (NSE) has been advocated as a useful marker for SCLC, it is also nonspecific because it stains up to 80% of NSCLC and rises in a considerable number of patients (20-30%) with NSCLC (3).

In recent years, a precursor of the neuropeptide gastrin-releasing peptide, progastrin-releasing peptide (ProGRP), has been reported as the most promising marker for SCLC, demonstrating sensitivity and specificity higher than NSE (4-6). Some authors (6) have emphasized the clinical value of ProGRP as the tumor marker of choice in SCLC. However, limited and inconsistent data are available on the prognostic value of this marker in lung cancer (7-9).

The purpose of this study was to investigate the diagnostic and prognostic significance of ProGRP in NSCLC and SCLC and compare this marker with other known markers in lung cancer such as CEA, CYFRA 21-1 and NSE.

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Patients and Methods

This study was performed in a prospective fashion on 162 consecutive patients between 1 November 2000 through 30 October 2004. There were 37 patients with benign pulmonary disease (BPD) (25 males, 12 females), 88 patients with NSCLC (63 males, 25 females) and 37 patients with SCLC (28 males, 9 females). The median age was 61 years (range: 32-81) for BPD, 62 years (range: 34-88) for NSCLC and 68 years (range: 32-81) for SCLC. Diagnoses of all lung tumors were confirmed pathologically. According NSCLC histological classification (10), 18 patients had squamous cell carcinoma (SQC), 32 patients had adenocarcinoma (AC), 23 patients large cell carcinoma (LCC) and 15 patients had unclassified NSCLC (UC) subtypes. NSCLC and SCLC patients were staged according to TNM classification (11) and the Veterans Administration Lung Cancer Group A staging System (12), respectively. Among NSCLC patients, 12 had stage 3A, 35 stage 3B and 41 stage 4. All NSCLC patients had inoperable disease. Among the patients with SCLC, 13 had limited and 24 had extensive disease. Serum samples were obtained after informed consent from all patients before the start of treatment and stored at –80˚C until analysis was performed.

Marker evaluation. Serum CYFRA 21-1 and NSE levels were measured with commercially available ELSA-CYFRA 21-1 and ELSA-NSE kits, respectively, from CIS Bio International, Gif-Sur Yvette, France; CEA with a commercial kit from Abbott Diagnostics, North Chicago, IL, USA; serum ProGRP was measured with an ELISA kit manufactured by ALSI, Japan, and distributed in Europe by IBL-Hamburg, Germany.

Cut-off values. In this study, cut-off levels for CEA and CYFRA 21-1 established at 95% specificity in the group of patients with BPD were used. They were 4.7 ng/ml and 3.2 ng/ml, respectively, as had been reported earlier (13). For NSE and ProGRP in the diagnostic setting, we used the cut-off points corresponding to 95% specificity in the BPD group, while in the prognostic setting, we applied cut-off points representing the median. The institutional normal ranges were used for: serum lactic dehydrogenase (LDH) ≤620 U/l and serum hemoglobin (Hgb) ≤12 g/dl.

Statistical analysis. For statistical analysis, all data were presented as medians and interquartile range (IQR). Non-parametric tests were used for comparisons between numeric variables. The Kruskal-Wallis one-way analysis of variance (ANOVA) was performed to test for overall homogeneity. Differences in numerical variables between categorical factors were tested by the Mann-Whitney test. Associations between categorical variables were evaluated with Fisher's exact test or chi-square test. For analyses of the sensitivity-specificity relation of the assay, receiver operating characteristic (ROC) curves were constructed and used as a tool for determination of an optimal cut-off value. Survival was measured from the time of diagnosis to the last follow-up evaluation or death. In the analysis of the prognostic impact of various factors, univariate survival analyses were performed by the Kaplan–Meier method and the log-rank test. Variables statistically significant ($p<0.05$) in the univariate steps were entered into multivariate Cox regression models (14). Statistical calculations were performed using SPSS for windows, version 10 (SPSS Inc., Chicago, IL, USA). A value of $p<0.05$ was considered significant.

Table I. *Distribution of NSE and ProGRP in patients with lung cancer.*

		Tumor marker, median (interquartile range)	
	N	NSE (ng/ml)	ProGRP (pg/ml)
Benign pulmonary disease	37	7.7 (6.0-11.0)	17 (13-29)
Non-small cell lung cancer			
Total	88	7.8 (5.6-12.9)	19 (12-30)
Age (years)		$p=0.53$	$p=0.87$
≤62	45	8.0 (6.0-13.9)	19 (14-30)
>62	43	7.7 (5.1-11.6)	20 (12-30)
Gender		$p=0.52$	$p=0.32$
M	63	8.0 (5.5-13.8)	21 (12-30)
F	25	7.4 (5.8-10.4)	17 (13-23)
PS		$p=0.51$	$p=0.85$
0-1	47	8.6 (5.5-15.1)	21 (13-30)
≥2	41	7.4 (5.6-10.9)	17 (12-29)
Histological type		$p=0.96$	$p=0.85$
Squamous	18	9.6 (4.8-18.5)	19 (11-27)
Adeno-	32	7.1 (5.7-11.4)	22 (13-31)
Large	23	7.9 (6.6-11.6)	18 (14-23)
Unclassified	15	8.0 (5.1-15.1)	23 (12-29)
TNM Stage		$p=0.11$	$p=0.01$
M0	47	7.0 (5.4-11.2)	16 (10-23)
M1	41	9.6 (6.3-15.3)	25 (16-32)
Small cell lung cancer			
Total	37	22.0 (14.1-73.4)	138 (55-1180)
Age (years)		$p=0.13$	$p=0.78$
≤68	17	16.5 (11.2-36.6)	298 (67-884)
>68	20	33.3 (15.1-83.9)	109 (46-2136)
Gender		$p=0.79$	$p=0.99$
M	28	21.6 (13.7-81.1)	209 (52-1320)
F	9	11.8 (28.9-51.6)	138 (56-1021)
PS		$p=0.05$	$p=0.14$
0-1	15	15.3 (11.0-34.6)	72 (19-360)
≥2	22	33.2 (16.2-153.5)	453 (74-1243)
Stage		$p=0.04$	$p=0.28$
Limited	13	15.0 (10.7-31.2)	91 (41-560)
Extensive	24	34.5 (15.6-106.3)	250 (68-1802)

Results

Tumor marker distribution in NSCLC. The lower detection limit for ProGRP assay, calculated from the standard curve as mean absorption of the zero standard (n=5) plus 3 SD, corresponded to 2 pg/ml. NSE and ProGRP values did not differ significantly between advanced NSCLC and BPD ($p=0.97$ and $p=0.13$). There was no association of either marker with age, gender, performance status (PS) or tumor histology (Table I). The difference in marker levels between advanced locoregional (stage 3) and metastatic (stage 4)

disease was significant for ProGRP (p=0.01), but not for NSE (p=0.11). In 71 out of 88 patients with NSCLC, the histological grade was reported. Only ProGRP demonstrated an association with grade, being significantly higher in patients with low-grade compared to those with high-grade tumors (Grade 1-2: n=35, median 24, IQR: 16-35 *versus* Grade 3-4: n=36, median 16, IQR: 9-23; p=0.002).

Tumor marker distribution in SCLC. NSE and ProGRP serum levels were significantly higher in SCLC than in BPD (for both p<0.0001). There was no significant difference of any marker levels according to the age, gender or performance status (Table I). Only NSE showed association with disease stage, being significantly higher in patients with extensive as compared to limited disease (median, 34.5 ng/ml and 15 ng/ml, respectively, p=0.04).

Analysis of ROC curves. To compare the ability of ProGRP and NSE to discriminate SCLC from BPD and NSCLC, the ROC curves were constructed (Figure 1a and 1b). The areas under the ProGRP and NSE ROC curves (± standard error) in the two models considering SCLC *versus* BPD, and SCLC *versus* NSCLC were: 0.90±0.04 and 0.87±0.04, and 0.89±0.04 and 0.84±0.04, respectively. Although there was no significant difference between the areas under the ProGRP and NSE ROC curves, at high specificity above 90%, the ProGRP assay demonstrated better characteristics than did the NSE assay. At cut-offs corresponding to 95% specificity in BPD (ProGRP: 48 pg/ml and NSE: 22 ng/ml), ProGRP sensitivity was significantly higher than that of NSE (78.4% and 48.6%, p=0.001, Table II). At 97.7% specificity for SCLC *versus* NSCLC, the ProGRP and NSE assays demonstrated sensitivity of 75.7% and 37.8%, respectively (p=0.001).

Sensitivity of markers by histology and disease stage. The analysis of marker sensitivity according to histology of advanced NSCLC (Table II) showed the highest sensitivity for CEA in AC (68.8%), for CYFRA 21-1 in SQC (83.3%) and equal sensitivity of these two markers in LC (43.5%). The overall sensitivity of CYFRA 21-1 in NSCLC was significantly higher than that of CEA (68.2% *vs*. 47.7%; p=0.01). Combined use of CYFRA 21-1 and CEA improved sensitivity up to 80%. NSE sensitivity in different histological subtypes of NSCLC ranged from 6.3% to 22.2%, with an overall sensitivity of 11.4%. ProGRP showed the lowest sensitivity (4.5%). Concerning tumor extension, *i.e*. disease stage, a significantly higher sensitivity in stage 4 compared to stage 3 disease was found for CEA (63.4% *vs*. 34.1%; p=0.01) and CYFRA 21-1 (92.7% *vs*. 46.8%; p<0.0001), but not for NSE (12.8% *vs*. 9.8%; p=0.7), nor for ProGRP (7.3% *vs*. 2.1%; p=0.3). There were only two patients in the NSCLC group who had ProGRP levels above 100 pg/ml (383 pg/ml and 583 pg/ml). These

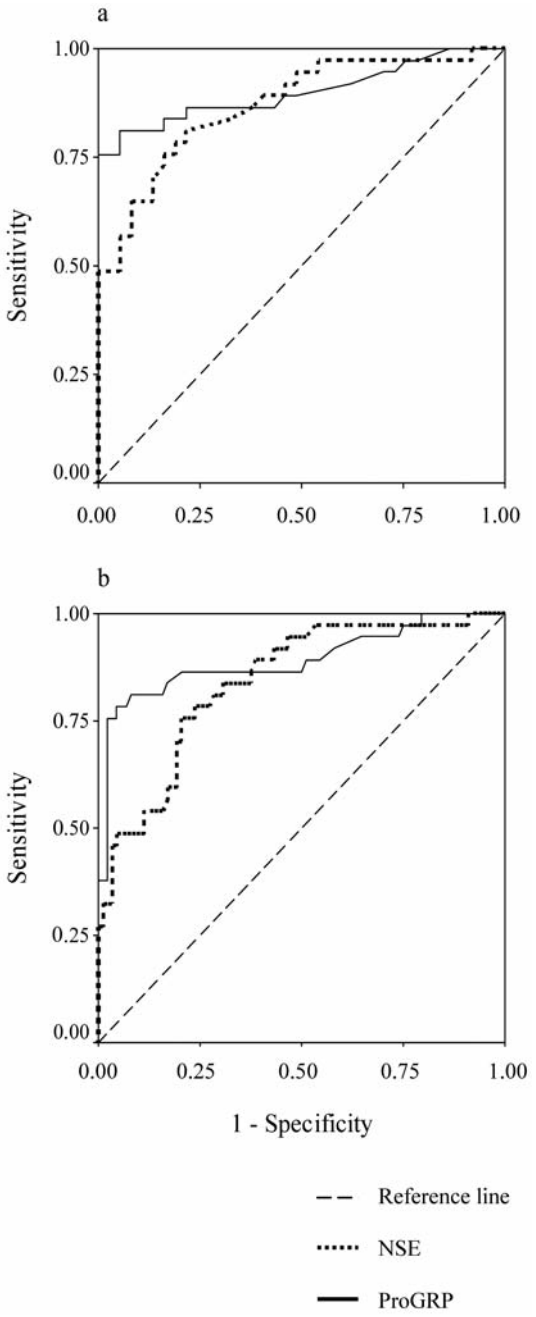

Figure 1. *Receiver operating characteristic curves for discrimination of SCLC from BPD (a) and NSCLC (b). NSCLC: Non-small cell lung carcinoma; SCLC: small cell lung carcinoma; BPD: benign pulmonary disease.*

patients had LCC, one of which was characterized as large cell neuroendocrine carcinoma (LCNEC).

In the SCLC group, no marker showed significant difference in sensitivity between extensive and limited disease (CEA, 46.2% *vs*. 45.8%, p=1.0; CYFRA 21-1, 50% *vs*. 38.5%, p=0.73; NSE, 30.8% *vs*. 69.2%, p=0.17; and ProGRP, 79.2% *vs*. 76.9%, p=1.0.

Table II. *Sensitivity of markers by histology of lung cancer.*

		Positive/total (sensitivity, %)					
Marker	Cut-off	AC	SQC	LCC	UC	NSCLC	SCLC
CEA	4.7 ng/ml	22/32 (68.8)	4/18 (22.2)	10/23 (43.5)	6/15 (40.0)	42/88 (47.7)	17/37 (45.9)
CYFRA 21-1	3.2 ng/ml	24/32 (75.0)	15/18 (83.3)	10/23 (43.5)	11/15 (73.3)	60/88 (68.2)	17/37 (45.9)
NSE	22 ng/ml	2/32 (6.3)	4/18 (22.2)	2/23 (8.7)	2/15 (11.4)	10/88 (11.4)	18/37 (48.6)
ProGRP	48 pg/ml	1/32 (3.1)	0/18 (0)	2/23 (8.7)	1/15 (6.7)	4/88 (4.5)	29/37 (78.4)

NSCLC: Non-small cell lung carcinoma; AC: adenocarcinoma; SQC: squamous cell carcinoma; LCC: large cell carcinoma; UC: unclassified NSCLC; SCLC: small cell lung carcinoma.

Survival. For NSCLC, the median survival (MS) significantly correlated with PS (PS 0-1 *vs*. ≥2, 13.1 months *vs*. 5.7 months; p=0.002), levels of CYFRA 21-1 (≤3.2 ng/ml *vs*. >3.2 ng/ml, 14.3 months *vs*. 8.1 months; p=0.03), ProGRP (≤19 pg/ml *vs*. >19 pg/ml, 8.6 months *vs*. 12.4 months; p=0.03, Figure 2a) and serum Hgb (>12 g/dl *vs*. ≤12 g/dl, 11.4 months *vs*. 7.4 months; p=0.04). The correlation of age, gender, stage, histology, LDH, CEA and NSE with survival was not significant. All significant variables were included in a multivariate analysis in which PS and CYFRA 21-1 remained significant predictors of survival, whereas ProGRP and Hgb were not significant explanatory variables (Table III).

For SCLC, statistically significant association with survival was observed for PS (PS 0-1 *vs*. ≥2, 13.4 months *vs*. 5.4 months; p=0.04), CYFRA 21-1 (≤3.2 ng/ml *vs*. >3.2 ng/ml, 14.1 months *vs*. 4.7 months; p=0.003) and LDH (≤620 U/l *vs*. >620 U/l, 14.2 months *vs*. 4.4 months; p=0.005). Age, gender, stage, CEA and NSE were not correlated to survival. At dichotomization of the SCLC group by applying the ProGRP median (138 pg/ml), the difference in MS was not significant (p=0.58). However, when a higher cut-off was used, the difference became significant (Figure 2b, ≤800 pg/ml *vs*. >800 pg/ml, 13 months *vs*. 6.3 months; p=0.042). The Cox model defined PS, CYFRA 21-1 and LDH as important characteristics with independent impact on survival, while ProGRP was not a significant variable (Table III).

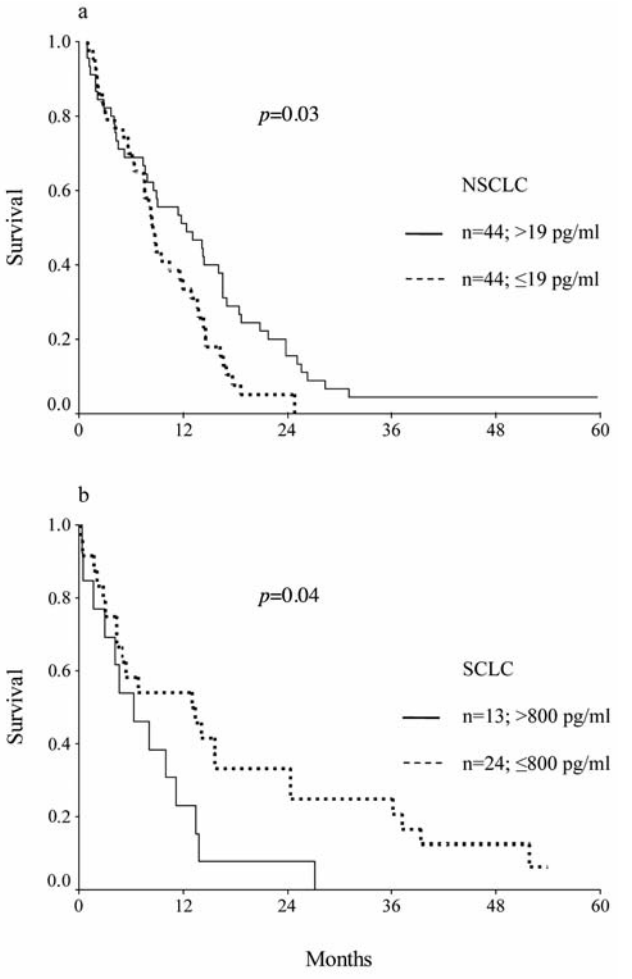

Figure 2. *Receiver operating characteristic (ROC) curves for discrimination of SCLC from BPD (a) and NSCLC (b). NSCLC: Non-small cell lung carcinoma; SCLC: small cell lung carcinoma; BPD: benign pulmonary disease.*

Discussion

Many biological elements inherent in the tumor cell, including CEA, CYFRA 21-1 and NSE, have been evaluated for their potential in the diagnosis and prognosis of lung cancer. In accordance with other data, we found that in advanced stage (III-IV), leading markers in AC and SQC were CEA and CYFRA 21-1, respectively (15). The highest sensitivity in NSCLC was found for CYFRA 21-1, reaching 68%. Combined use of two markers improved sensitivity to 80%. The results of our study have confirmed that ProGRP is more useful than the NSE marker for the diagnosis of SCLC (6, 15-17). At cut-offs corresponding to 95% specificity in the BPD group (48 pg/ml), the sensitivity of ProGRP was 78.4%, while that of NSE was only 48.6%.

Table III. *Multivariate Cox's regression analysis: estimated relative risk.*

Characteristics	Patients, n	Relative risk	95% CI	*p*-Value
Non-small cell lung cancer				
PS				
0-1	47	1		
≥2	41	1.9	1.2-3.1	0.005
CYFRA 21-1 (ng/ml)				
≤3.2	28	1		
>3.2	60	1.8	1.1-2.8	0.020
ProGRP (pg/ml)				
≤19	44	1		
>19	44	0.7	0.4-1.1	0.100
Hemoglobin				
>12 g/dl	65	1		
≤12 g/dl	23	1.5	0.9-2.6	0.120
Small cell lung cancer				
PS				
0-1	15	1		
≥2	22	2.4	1.2-5.2	0.020
CYFRA 21-1 (ng/ml)				
≤3.2	20	1		
>3.2	17	2.6	1.2-5.3	0.010
LDH (U/l)				
≤620	17	1		
>620	20	3.0	1.4-6.5	0.005
ProGRP (pg/ml)				
≤800	24	1		
>800	13	1.6	0.8-3.8	0.200

95% CI: 95% Confidence interval; PS: performance status; LDH: lactic dehydrogenase.

Previous studies had reported sensitivity for ProGRP between 47% and 86% at cut-offs ranging from 33.8 pg/ml to 53 pg/ml (4, 5, 8, 16-18).

Although NSE has been recognized as a marker for SCLC, a considerable number of NSCLC patients in our study (11.4%) also had elevated levels of this marker. We found that the ProGRP assay showed clinical performance better than that for NSE in discriminating between these two main lung cancer entities, especially at a specificity of above 90%. At 97.7% specificity for SCLC *versus* NSCLC, the ProGRP assay showed a sensitivity of 75.7%, while the NSE assay only 37.8%. Only two patients with high ProGRP levels >100 pg/ml were found in the NSCLC group. Both of them had LCC. One of these tumors was diagnosed as LCNEC. These findings are in accord with results of Goto *et al*. (19), who emphasized that NSCLC patients with ProGRP levels above 100 pg/ml should be examined for the presence of the small cell component or neuroendocrine differentiation. On the other hand, ProGRP may well be a candidate marker for all pulmonary neuroendocrine neoplasms, comprising the carcinoids, LCNEC and SCLC (20), and applicable mainly to their higher grade variants.

The univariate survival analysis in NSCLC demonstrated a significant association of MS with PS, serum CYFRA 21-1, Hgb and ProGRP. Results of this study have confirmed our previous observation (21) that patients with low levels of ProGRP (<19 pg/ml) have significantly shorter MS than those with relatively high levels. The reason for this finding may be related to some biological characteristics of NSCLC. Our results demonstrating a significant association of low ProGRP levels with high-grade tumors may support this line of reasoning. This finding may reflect a down-regulation of ProGRP production in NSCLC with its progression to more aggressive poorly differentiated tumors. In multivariate analyses, only PS and CYFRA 21-1 were found as significant variables, while ProGRP and Hgb, being controlled for PS and CYFRA 21-1, did not retain significance.

In SCLC, a significant association with survival was shown for PS, CYFRA 21-1 and LDH. The literature data on the prognostic value of ProGRP in SCLC do not seem to be consistent. The lack of any prognostic value of ProGRP has been reported in two studies performed on SCLC patients treated with cisplatin-based chemotherapy (7, 9), while in a third study, the patients with elevated ProGRP levels were shown to have a significantly shorter MS (8). In our study, at dichotomization of SCLC patients by ProGRP threshold representing the median (138 pg/ml), the difference in MS between the two sets of patients was not significant. However, when a higher cut-off value was applied (>800 pg/ml) for the categorization of SCLC patients, the difference did become significant.

ProGRP is a biologically active protein, stimulating proliferation of tumor cells (22). A high correlation between ProGRP and GRP has been observed in tissue extracts, suggesting that most ProGRP is processed to GRP (23). It was reported that GRP may function as an autocrine growth factor for SCLC (24). It appears that growth-stimulating properties of ProGRP may be responsible for more aggressive tumor behavior and shorter survival. Thus, two different mechanisms may explain the poor prognosis for NSCLC and SCLC patients with low and high serum ProGRP expression, respectively.

Our study confirms the previous data on CYFRA 21-1 as an important prognostic factor not only in NSCLC, but in SCLC as well (9, 25, 26). This tumor marker was shown to retain significance in a multivariate survival model along with PS and LDH.

Our study supports ProGRP as an important marker of SCLC and points the way for further clinical studies to explore its value in monitoring response to therapy and patients' follow-up.

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