ANTICANCER RESEARCH *24*: 1941-1946 (2004)

The Prognostic Value of Both Neuron-specific Enolase (NSE) and Cyfra21-1 in Small Cell Lung Cancer

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Abstract. *Purpose: The aim of this study was to verify the prognostic significance of multiple tumour markers in small cell lung cancer (SCLC). Patients and Methods: We examined seven tumour markers [carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA19-9), squamous cell carcinoma antigen (SCC), neuron-specific enolase (NSE), cancer antigen 125 (CA125), cytokeratin 19 fragment (Cyfra21-1) and Pro-Gastrin-releasing peptide (ProGRP)] in 57 small cell lung cancer (SCLC) patients. Results: Univariate analysis showed that NSE and Cyfra21-1 were independent negative prognostic factors along with gender, therapy and lactate dehydrogenase (LDH). Multivariate analysis showed that both NSE and Cyfra21-1 retained their significance as prognostic factors along with therapy and the respective hazard ratios were 3.918 (p=0.0122) and 2.617 (p=0.0318) among the seven tumour markers. The group with both NSE and Cyfra21-1 positive had a worse prognosis than the only NSE-positive group, with the respective hazard ratios being 10.245 (p=0.0004) and 3.913 (p=0.0123). Conclusion: The group with both of the markers NSE and Cyfra21-1 positive had a worse prognosis than the only NSE-positive group.*

In small cell lung cancer (SCLC), there have been several reports concerning tumour markers and prognosis but no marker has been shown to have a reproducible prognostic significance (1-9). These reports examined tumour markers for many cases (maximum included 263 patients: Ref. 7), but without reaching clear consensus. Also, cancer antigen 19-9 (CA19-9), squamous cell carcinoma antigen (SCC) and cancer antigen 125 (CA125) have never been investigated as possible prognostic markers of SCLC in previous reports. The aim of this study was to verify the prognostic significance of multiple tumour markers, including CA19-9, SCC and CA125, in SCLC patients.

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Key Words: NSE, Cyfra21-1, multiple tumour markers, small cell lung cancer, prognosis.

Patients and Methods

Patients. Between June 1996 and September 2003, according to our medical records, 57 patients had had pathological or cytological diagnoses of SCLC; subjects for this retrospective study were selected from these patients (Table I). Patients with non-small cell carcinoma, with active tumours of other organs and with lung diseases that might affect tumour markers (such as interstitial pneumonia), were excluded. Patients who had been treated in other hospitals prior to the consultation at our hospital were also excluded. A pathological or cytological diagnosis of SCLC was obtained using bronchofiberoscopy, mediastinoscopy, surgical resection, or biopsy of lymph nodes. A performance status was estimated according to the Eastern Cooperative Oncology Group scale. The diagnosis and stage of the cases were decided by means of bronchofiberoscopy, computed tomography (CT) scans, magnetic resonance imaging (MRI), mediastinoscopy, surgical lymph node resection with histological examination, ultrasonography of the abdomen, radioisotope examination (Ga, Tl and Tc bone scans) or both biopsy and aspiration of bone marrow. The staging was based on the TNM classification of the International Union Against Cancer (10) and the U.S. Veterans Administration Lung Cancer Group (11). According to the above, the stages of the SCLC patients were classified into the following two groups: 1) limited disease (LD), a disease confined to one hemithorax including mediastinal lymph nodes and/or supraclavicular lymph nodes; 2) extensive disease (ED), defined as having the opposite criteria to LD. Using this classification, the SCLC patients were identified as 37 LD and 20 ED.

Tumour markers. We examined seven tumour markers [carcino-embryonic antigen (CEA), cancer antigen 19-9 (CA19-9), squamous cell carcinoma antigen (SCC), neuron-specific enolase (NSE), cancer antigen 125 (CA125), cytokeratin 19 fragment (Cyfra21-1) and Pro-Gastrin-releasing peptide (ProGRP)] in patients at their first visit to our hospital. All patients gave their consent to having blood samples taken and this retrospective study was approved from the standpoint of ethics by the Institutional Review Board. CEA and CA19-9 were assayed with a chemiluminescence immunoassay kit (Abbott Laboratories, Illinois, USA) and SCC by an enzyme immunoassay kit (Abbott Laboratories). NSE and CA125 were assayed using a solid-phase radioimmunoassay kit (NSE; Eiken Chemical Co.Ltd., Tokyo, Japan, CA125; Centocor, Inc. Malvern, PA, USA) according to the one-step sandwich assay. Cyfra21-1 levels were determined using an

Table I. *Patient profiles.*

	Number of patients
Total	57
Gender (male/female)	48/9
Mean age (range)	66 (48-78)
Stage (LD/ED)	37/20
PS0-1/2-3	51/6
Smoking (pack-year)	66 (5-175)
Therapy	
Chemotherapy/Radiation	24
Chemotherapy	16
Operation	11
Radiation	1
None	4
Other	1

electrochemoluminescence immunoassay kit (Roche Diagnostics GmbH, Mannheim, Germany) by the two-step sandwich method. ProGRP was assayed with an enzyme-linked immunoadsorbent assay kit (TFB. Inc., Tokyo, Japan) by the two-step sandwich method. To determine the cut-off level of the tumour markers, the serum level of each was measured in 76 patients with benign lung diseases (26 mycobacterial infections, 4 organizing pneumonia cases, 4 intrapulmonary lymph node cases, 5 lung abcess, 3 sarcoidosis, 2 sequestration cases, 2 Aspergillomas, 1 pulmonary dilophilariasis, 7 other infections without evident organisms, 13 hamartomas, 2 sclerosing pneumocytomas, 2 sugar tumours, 1 inflammatory pseudotumour, 1 inflammatory polyp, 3 other lung diseases cases).

Statistical analysis. The Mann-Whitney *U*-test was used to compare the tumour markers with clinical factors. Survival times were calculated as intervals from the date of diagnosis to that of death or the date of the last contact. Patients were censored at the last date of contact if they survived. A survival analysis was performed by the Kaplan-Meier method (12). To evaluate the prognostic significance, we carried out univariate analysis using the log-rank test (13) for other clinical factors including the disease extent and performance status (PS), generally regarded as prognostic factors, and a multivariate analysis using Cox's proportional hazard model (14). Also, the patients were divided into two groups: 1) therapy done, the patients who underwent the curative or systemic therapy and 2) therapy none, the patients who had only palliative therapy. A p value of 0.05 or less was considered significant for all tests. For statistical analysis, we used Stat View J 5.0 software (SAS Institute Inc., USA).

Results

Serum tumour marker values. The serum levels of all tumour markers except CA 19-9 were significantly elevated in SCLC patients compared with benign disease patients (Table II). We established the cut-off levels of tumour markers from the serum marker levels of patients with benign pulmonary diseases according to a previous report (mean+3 x standard deviation) (3). The cut-off values of tumour markers were 8.3 ng/ml for CEA, 53.6 U/ml for CA19-9, 1.5 ng/ml for SCC, 10.5 ng/ml for NSE, 53.0 U/ml for CA125, 2.4 ng/ml for Cyfra21-1 and 40.1 pg/ml for ProGRP. The upper normal limits of other variables were as follows: lactate dehydrogenase (LDH): 200 IU/l; alkaline phosphatase: 330 IU/l. The normal range of the serum sodium was 135-145 mEq/l. Based on these cut-off levels, the total positive rates of tumour markers are listed in Table III.

Serum levels of tumour markers and stages. Serum NSE, CA125 and Cyfra21-1 levels were significantly elevated in the ED group compared with the LD group in SCLC patients (Table IV). Other marker levels were not correlated with the stage in SCLC patients.

Prognostic values of tumour markers. The median observation time was 406 days for survival analysis in the censored patients. Univariate analysis showed that gender, therapy, LDH, NSE and Cyfra21-1 were significant prognostic factors (Table V). No prognostic significance of other tumour markers or clinical factors were observed. Using these five significant factors as covariates, multivariate analysis showed that therapy, NSE and Cyfra21-1 retained their significance as prognostic factors, with the hazard ratios being 0.057 (p=0.0003), 3.918 (p=0.0122) and 2.617 (p=0.0318), respectively (Table VI). In the patients with positive NSE levels, median survival time was 635 days, while in those with negative NSE it was 229 days (Figure 1a). In patients with positive NSE, the survival rates were 40.3% at 1 year and 14.8% at 2 years, respectively, while in those with negative NSE, the survival rates were 89.5% at 1 year and 49.7% at 2 years, respectively (Figure 1a). In the patients with positive Cyfra21-1 levels, median survival time was 408 days, while in those with negative Cyfra21-1, that time was 165 days (Figure 1b). In patients with positive Cyfra21-1, the survival rates were 28.0% at 1 year and 9.3% at 2 years, respectively, while in those with negative Cyfra21-1, the survival rates were 65.8% at 1 year and 32.1% at 2 years, respectively (Figure 1b). Thirteen out of 14 patients with elevated serum levels of Cyfra21-1 also had elevated serum levels of NSE. Cyfra21-1 could depict the worse prognosis among the NSE-positive patients. We divided the patients into three groups: group A, patients with NSE and Cyfra21-1 both negative; group B, patients with only NSE-positive; and group C, patients with both positive (excluded one patient with the only Cyfra21-1-positive) (Figure 1c). The hazard ratio of group C in comparison with group A was 10.245 (Table VII). The hazard ratio of group B was 3.913 (Table VII). Although there was no significant difference between group C and group B (p=0.1031), group C had a worse prognosis than group B (median survival times were 187 and 255 days, respectively, the survival rates were 25.0% and 46.3% at 1 year, 8.3% and 13.2% at 2 years, respectively).

Table II. *Differences in serum levels of tumour markers between SCLC and benign disease patients.*

	CEA	CA19-9	SCC	NSE	CA125	Cyfra21-1	ProGRP
benign disease	2.3(2.0)	11.3 (14.1)	0.6 (0.3)	6.3 (1.4)	14.9 (12.7)	1.2 (0.4)	18.2 (7.3)
SCLC	8.1 (14.4)	20.5 (56.2)	0.7 (0.4)	27.7 (28.7)	40.8 (82.3)	2.0 (1.2)	667.2(1519.7)
p value	<0.0001*	0.7124	0.0492*	<0.0001*	0.0357*	<0.0001*	<0.0001*

mean level (standard deviation)
* Mann-Whitney *U*-test

Table III. *Positive rates of tumour markers in SCLC patients.*

	CEA	CA19-9	SCC	NSE	CA125	Cyfra21-1	ProGRP
Positive rate (%)	19.3	8.8	5.3	63.2	15.8	24.6	64.9

Table IV. Differences in serum levels of tumour markers between clinical stages in SCLC patients.

	CEA	CA19-9	SCC	NSE	CA125	Cyfra21-1	ProGRP
LD	6.7 (10.0)	10.6 (18.6)	0.7 (0.3)	21.0 (23.9)	27.8 (58.4)	1.6 (0.7)	466.5 (986.2)
ED	10.7 (20.1)	38.9 (90.1)	0.6 (0.5)	40.2 (33.0)	64.8 (112.0)	2.7 (1.5)	1038.5 (2177.3)
p value	0.1600	0.0905	0.5322	0.0029*	0.0127*	0.0008*	0.1921

mean level (standard deviation)
* Mann-Whitney *U*-test

Discussion

Several reports have been published about the negative prognostic value of tumour markers, for example NSE (1, 3-5), Cyfra21-1 (2, 4), tissue polypeptide-specific antigen (TPS) (8) and Chromogranin A (4, 9). However, to our knowledge, no reports about the prognostic value of a combination of NSE and Cyfra21-1 have been previously published for SCLC. NSE is a well-established tumour marker for SCLC and is produced by other neuroendocrine tumours too. Cyfra21-1 is a specific and reproducible negative-prognostic marker for non-small cell lung cancer (NSCLC) (6, 15-23). In our study, most of the patients with elevated levels of serum Cyfra21-1 also had elevated levels of serum NSE. NSE-positive patients were divided into two groups which had different prognoses dependent on the positive or negative status of Cyfra21-1. The group positive for both NSE and Cyfra21-1 had a worse prognosis than the only NSE-positive group. Cyfra21-1 is thought to have an additional value to NSE from a prognostic point of view.

Table V. *Univariate multivariate analyses of clinical factors and tumour markers.*

Univariate analyses	χ^2	*p* value
Gender (male *vs* female)	4.725	0.0297*
Age (69≤ *vs* 69>)	2.254	0.1333
Stage (ED *vs* LD)	1.709	0.1911
Smoking (pack-year 60≤ *vs* 60>)	1.546	0.2137
PS (0-1 *vs* 2≤)	0.615	0.4330
Therapy (done *vs* none)	22.453	<0.0001*
LDH (200 IU/l≤ *vs* 200 IUl>)	8.147	0.0043*
ALP (330 IU/l≤ *vs* 330 IUl>)	2.326	0.1272
Sodium (135 mEq/l≤ *vs* 135 mEql>)	1.680	0.1950
CEA (8.3 ng/ml≤ *vs* 8.3 ng/m>)	1.701	0.1922
CA19-9 (53.6 U/ml≤ *vs* 53.6 U/m>)	2.925	0.0872
SCC (1.5 ng/ml≤ *vs* 1.5 ng/m>)	0.023	0.8806
NSE (10.5 ng/ml≤ *vs* 10.5 ng/m>)	13.942	0.0002*
CA125 (53.0 U/ml< *vs* 53.0 U/m≥)	0.009	0.9258
Cyfra21-1 (2.4 ng/ml< *vs* 2.4 ng/m≥)	11.655	0.0006*
ProGRP (40.1 pg/ml< *vs* 40.1 pg/m≥)	2.521	0.1123

Table VI. *Multivariate analyses of clinical factors and tumour markers.*

Multivariate analyses	Hazard ratio	95%CI	*p* value
Gender (male *vs* female)	0.510	0.136-1.917	0.3189
Therapy (done *vs* none)	0.057	0.012-0.265	0.0003*
LDH (200 IU/l≤ *vs* 200 IUl>)	1.567	0.608-4.039	0.3525
NSE (10.5 ng/ml≤ *vs* 10.5 ng/m>)	3.918	1.347-11.394	0.0122*
Cyfra21-1 (2.4 ng/ml< *vs* 2.4 ng/m≥)	2.617	1.087-6.302	0.0318*

CI = confidence interval

Table VII. *Multivariate analyses including the combination of NSE and Cyfra21-1.*

Multivariate analyses	Hazard ratio	95%CI	*p* value
Gender (male *vs* female)	0.510	0.136-1.915	0.3181
Therapy (done *vs* none)	0.057	0.012-0.266	0.0003*
LDH (200 IU/l≤ *vs* 200 IUl>)	1.567	0.608-4.039	0.3524
Both NSE and Cyfra (positive *vs* negative)	10.245	2.826-37.146	0.0004*
Only NSE (positive *vs* negative)	3.913	1.345-11.386	0.0123*

CI = confidence interval

Other clinical factors which have been reported as prognostic factors are performance status, disease extent, age, gender, LDH, alkaline phosphatase and serum sodium, but, above all, performance status and disease extent are two well-established predictive factors (24-26). We failed to demonstrate any prognostic value for both performance status and disease extent. There is also some controversy about the prognostic significance of these factors: performance status [significant (1, 4, 5, 7, 9, 24, 25), not significant (3, 26)], disease extent [significant (7, 9, 24, 26), not significant (3, 4, 8)]. These controversies may indicate the heterogeneity of SCLC. CA19-9, SCC and CA125 have never been investigated as prognostic factors of SCLC. We examined seven tumour markers including these three markers and we could not show their prognostic significance.

In conclusion, among seven tumour markers, both NSE and Cyfra21-1 had a negative prognostic significance and the group with both markers positive had a worse prognosis than the only NSE-positive group. Cyfra21-1 had an additional prognostic value to NSE in SCLC patients.

Acknowledgements

The authors are grateful to Mrs. Michiko Ando (Department of Chest Medicine, Graduate School of Medicine, Chiba University, Japan) and Naomichi Iwai (Division of Thoracic Surgery, Matsudo Municipal Hospital, Japan) for their assistance in the preparation of this manuscript.

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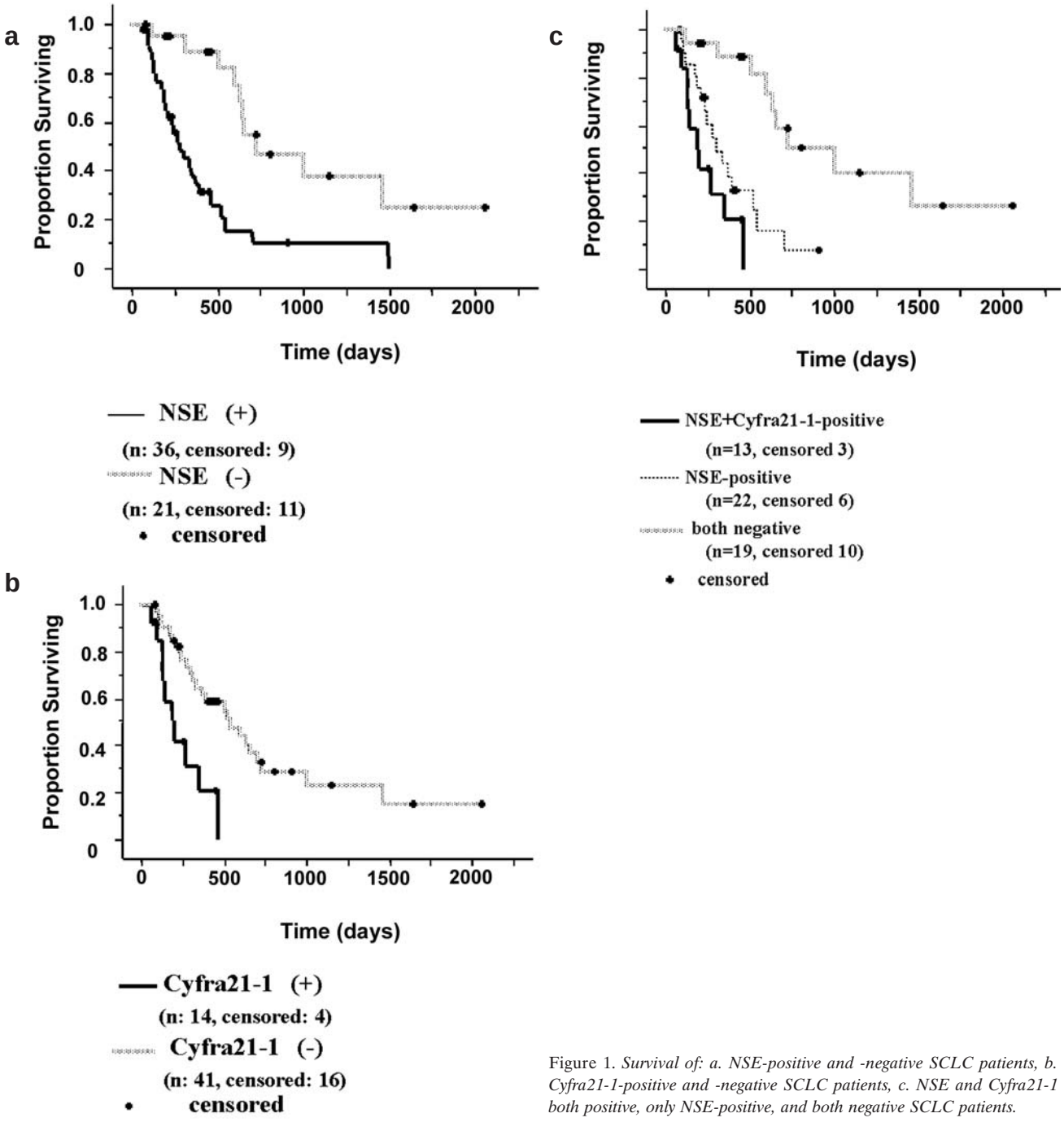

Figure 1. *Survival of: a. NSE-positive and -negative SCLC patients, b. Cyfra21-1-positive and -negative SCLC patients, c. NSE and Cyfra21-1 both positive, only NSE-positive, and both negative SCLC patients.*

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Received December 29, 2003
Revised February 3, 2004
Accepted April 12, 2004