

CYFRA 21-1 and CEA are Independent Prognostic Factors in 153 Operated Stage I NSCLC Patients

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Abstract. Currently, no further therapy in addition to surgery is recommended in completely resected NSCLC stage I patients. However, the 5-year survival rate at this stage has been reported to be approximately 60%, i.e. 40% of patients had a lower survival rate. The aim of the study was to identify those patients at increased risk by using the tumor markers CYFRA21-1 and CEA as prognostic factors. One hundred and fifty-three stage I NSCLC patients, who were treated exclusively by surgery between 1996 and 1998, entered this retrospective study. It was shown, by multivariate analysis, that elevated CYFRA 21-1 (>3.3ng/ml) and CEA (>9.8ng/ml) levels were associated with a worse outcome in 21.3% and 13.1% of the patients under study, respectively. The corresponding 3-year survival rates were found to be 60.2% for increased CYFRA 21-1 levels ($p=0.029$) and approximately 40% for increased CEA levels ($p=0.022$), compared to a rate of 78.4% and 79.0% in case of normal marker levels, respectively. The relative risk (95% confidence interval) was found to be 2.156 (1.08-4.29) for elevated CYFRA 21-1 and 2.707 (1.15-6.36) for elevated CEA. The detection rate for the identification of patients with worse outcome increased when a combination of both markers was used. Thereby, it was possible to identify 32% of patients where one or both markers were elevated. The 3-year survival rate was 55.7% in this group compared to that of 82.5% in those patients where both markers were in the normal range ($p=0.0014$). In order to consider the degree of marker elevation that is thought to reflect tumor burden, we introduced a tumor marker index (TMI)

corresponding to the geometric mean of normalized CYFRA21-1 and CEA levels (marker value divided by diagnostic cut-off). Thereby, we were able to identify 3 groups of patients at different risk levels: the first group (22.7%) had a 3-year survival rate of 96.7%, the second group (42.6%) had one of 77.2% and the third group (34.7%) had one of only 55.7%. In conclusion, elevated CYFRA 21-1 and CEA levels were able to identify a group of curatively operated NSCLC patients who were at high risk of early death. Those patients may benefit from more aggressive treatment approaches. The group of patients with a 3-year survival rate of 96.7% probably does not need further treatment.

Surgery is the treatment of choice in the early stages of non-small cell lung cancer (NSCLC). It is the only therapeutic option with the potential to cure. At present, no further therapy in addition to surgery is recommended in completely resected UICC stage I patients (1, 2).

The 5-year survival rate at this stage has been reported to be approximately 60%, i.e. the remaining 40% of patients had a lower survival rate. The latter patients might be candidates for an adjuvant treatment. One of the current challenges in NSCLC treatment is to improve the survival of curatively operated patients.

However, surgeons hesitate to assign completely resected stage I patients to adjuvant therapeutical schedules, because those patient are thought to be cured and would then be overtreated. Therefore, clearly there is a need for prognostic factors to exclusively identify those patients who are at an increased risk.

There is growing evidence that tumor markers provide prognostic information in NSCLC (3). It was shown that patients with elevated marker levels had an increased hazard ratio compared to patients with normal marker expression (4). However, most studies dealing with the prognostic value of tumor markers suffer from relatively small study populations and a broad heterogeneity in respect to tumor stage, histology and treatment (3 - 6).

Recently, we reported on the prognostic significance of the tumor markers CYFRA 21-1, CEA and NSE in a group

Abbreviations: NSCLC, non-small cell lung cancer; CYFRA 21-1, cytokeratin 19 fragment 21-1; CEA, carcinoembryonic antigen; NSE, neuron-specific enolase; LDH, lactate dehydrogenase; p-stage, postoperative stage; PS, performance status; ECOG, Eastern Cooperative Oncology Group; CI, confidence interval.

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Table I. Patient characteristics.

Male/ Female	121/32 (20.9%)
Mean age:	64.8 [32-78] years
Squamous cell carcinoma	59 (38.6%)
Adenocarcinoma	75 (49.0%)
Large cell carcinoma	10 (6.5%)
NSCLC (mixed cell types)	9 (5.9%)

of 515 patients treated with surgery alone or with surgery in combination with chemotherapy and/or radiotherapy (7).

By the use of multivariate Cox model, here we analyzed the prognostic impact of CYFRA 21-1 and CEA in completely resected stage I NSCLC patients who have been treated exclusively by surgery. The aim of the study was to identify those patients who are at an increased risk at p-stage I.

Materials and Methods

Patients. One hundred and fifty-three stage I NSCLC patients, treated exclusively by surgery at our institution between 1996 and 1998, entered the current retrospective study. The diagnosis of lung cancer was confirmed by pathological examination and classified according to World Health Organization criteria (8). The postoperative tumor stage was determined following the revised International System for Staging of Lung Cancer (9). Last actualization of survival data was June 2002. The characteristics of the study population are listed in Table I. One hundred and twenty-one patients were male, 32 were female. The mean age was 64.8 years. Lobectomy was the treatment most commonly performed in the study population (90.2%), followed by pneumonectomy (7.2%) and segmental resection (2.7%). All patients had a complete resection (R0). Fifty-one patients were in stage pT1 and 102 patients were in stage pT2. All patients had a good performance status according to the Eastern Cooperative Oncology Group (ECOG) classification.

Tumor-associated antigens. Blood specimens were drawn prior to therapy. The serum was kept frozen at -20°C until analysis. CYFRA 21-1, CEA and NSE were measured by commercially available enzyme immunoassays on a Cobas Core analyzer (Roche Diagnostics, Mannheim, Germany). The results of the marker measurements are shown in Table II. In 12 cases we had missing CYFRA 21-1 values. LDH was measured as a routine parameter on a Hitachi 912 analyzer (Roche Diagnostics). (Data not shown).

Statistical analysis. The evaluation of discriminatory values for preoperative biomarkers (CYFRA21-1, CEA, NSE, LDH) which differentiated best between groups of patients with good and poor prognosis was done with the critlevel procedure of Abel *et al.* (10) using ADAM statistical software package (German Cancer Research Center, DKFZ, Heidelberg, Germany). If no significant discriminatory value could be found by this method (*i.e.* NSE, LDH), the upper limit of the normal range of the respective parameter was applied as a cut-off point to define patient groups. Based on the cut-off points, the biological parameters were transformed into binary variables.

Table II. Percentiles of the results of tumor marker measurement (ng/ml).

	n	percentiles		
		5%	Median	95%
CYFRA 21-1	141	0.9	1.8	8.0
CEA	153	1.1	3.5	21.0
NSE	150	6.4	10.6	18.8

All the other statistical analyses were done using SPSS 9.0 for Windows (Chicago, IL, USA). Univariate analysis of survival data was performed according to Kaplan and Meier (11). Significance between patient groups was tested using the log-rank test. A *p*-value of less than 5% was considered significant. The correlation between significant factors of univariate survival analysis was tested with Spearmans rank correlation. Independent factors were considered in the multivariate analysis. For multivariate survival analysis, the Cox proportional hazard regression analysis was applied (12).

Tumor marker index. In order to normalize tumor markers, the concentration value of each marker was divided by the diagnostic cut-off point of the individual test, *i.e.* 3.3 ng/ml for CYFRA 21-1 and 5.0 ng/ml for CEA). Thereafter the geometric mean of the normalized marker values was assessed (square root of [CYFRA21-1 norm. X CEA norm.]) (Figure 1). The result of the latter calculation was then referred to as the tumor marker index (TMI). TMI was further categorized by the above mentioned critlevel procedure.

Results

The results of the univariate and multivariate analyses are summarized in Table III. The overall 3- and 5-year- survival rates in operated p-stage I were 75% and 58%, respectively. In univariate analysis, male patients and patients with an age of 70 years and older were found to have a poorer outcome. No significant differences were seen between patients with pT1 and pT2 tumors and, among the histological subtypes of squamous cell carcinoma, adenocarcinoma and large cell carcinoma.

Discriminatory values for CYFRA 21-1 and CEA, which differentiate between patient groups with good and poor prognosis, have been described previously (7). By applying a cut-off value of 3.3 ng/ml for CYFRA 21-1 and a cut-off value of 9.8 ng/ml for CEA, we could observe significant differences in survival rates between patients with marker values below and those with marker values above these critical values (Table III). The 3-year survival rate was 78.4% in patients with normal CYFRA 21-1 levels and 60.2% in those with elevated marker values (>3.3ng/ml, *p*=0.015). The corresponding values for CEA were 79.2% and 41.6%, respectively (>9.8ng/ml; *p*=0.014). The detection rates for the identification of patients with a poorer outcome were 21.3% for CYFRA 21-1 and 13.1% for CEA. When considering both markers, the sensitivity increased to 32%. The 3-year survival rate was 82.5% for

$$\text{TMI} = \sqrt[2]{\frac{\text{CYFRA 21-1 ng/ml}}{3.3 \text{ ng/ml}} \times \frac{\text{CEA ng/ml}}{5.0 \text{ ng/ml}}}$$

Figure 1. Formula for the calculation of the tumor marker index (TMI). TMI corresponds to the geometric mean of the normalized tumor markers CYFRA 21-1 and CEA levels. Normalization, i.e. "multiple of the upper limit of normal" was done by dividing marker values by the diagnostic cut-off of the individual marker assay e.g. 3.3 ng/ml for CYFRA 21-1 and 5.0 ng/ml for CEA.

Table III. Results of survival analysis.

	Univar	Multivar	Hazard ratio	95% CI
Gender	0.029	0.053	3.25	0.98-10.69
Age (≥ 70 years)	0.026	0.114	1.81	0.87-3.78
ECOG PS 0 vs 1	0.3	--	--	--
pT1 vs pT2	0.14	--	--	--
Histology (overall)	0.18	--	--	--
Adeno vs Squamous	0.5	--	--	--
LDH (240 U/L)*	0.38	--	--	--
NSE (14.5 ng/ml)*	0.71	--	--	--
CEA (9.8 ng/ml)#	0.014	0.022	2.71	1.15-6.36
CYFRA 21-1 (3.3 ng/ml)#	0.015	0.024	2.16	1.08-4.30

*diagnostic cut-off point (upper limit of the normal range)

#prognostic cut-off point (optimized by crit level procedure)

patients with both markers in the normal range, and dropped to 55.7% if one or both markers were elevated ($p=0.0014$).

No discriminatory values could be found for NSE and LDH. Therefore, the upper limit of the normal range i.e. 14.5 ng/ml (NSE) and 240 U/ml (LDH) were used as cut-off points. No significant differences in survival could be found for these parameters (Table III).

All factors which reached a p -value of at least 0.1 were tested for independence. There was no significant correlation among age, gender, CYFRA 21-1 and CEA levels. Therefore, these factors entered the multivariate analysis. Only CYFRA 21-1 and CEA retained significance in the final multivariate Cox model. The results of multivariate analysis and the corresponding hazard ratios are listed in Table III. Both CYFRA 21-1 and CEA proved to be independent prognostic factors in the multivariate Cox regression analysis. The relative risk was found to be 2.156 (95%CI: 1.08-4.29; $p=0.03$) in case of increased CYFRA 21-1 and 2.707 (95% CI: 1.15-6.36; $p=0.02$) in case of CEA.

In an attempt to combine both marker values into one single variable, the geometric mean of normalized CYFRA 21-1 and CEA was used (TMI) (Figure 1). In the analysis of TMI values using the critlevel procedure, we were able to find two discriminatory values at 0.48 and 0.83. By using

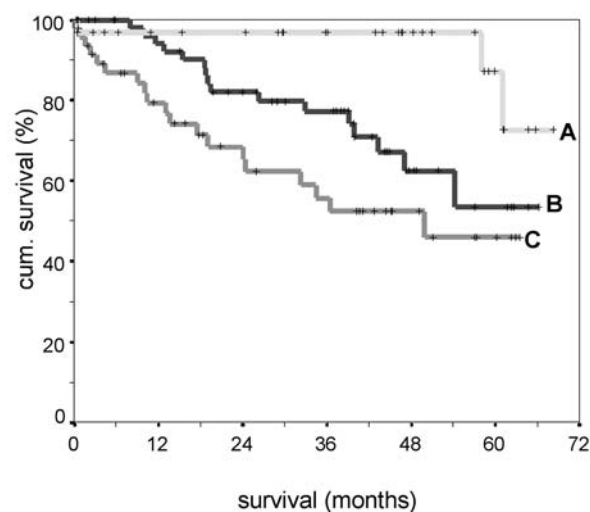


Figure 2. Survival in completely resected stage I NSCLC in respect to tumor marker index (TMI) based on CYFRA21-1 and CEA levels. A: TMI <0.48, n=32; B: TMI 0.48-0.83, n=60; C: TMI>0.83, n=49. A vs B $p=0.017$, A vs C $p=0.0008$, B vs C $p=0.077$ (log-rank test). + : censored data

these values we could clearly differentiate between three prognostic groups, one with a low ($n=32$, 22.7%), one with an intermediate ($n=60$, 42.6%) and one with a high ($n=49$, 34.7%) risk of early death (Figure 2). The 3-year survival rates were 96.7%, 77.2% and 55.7%, respectively.

Discussion

It could be shown by univariate and multivariate analyses that elevated pretreatment CYFRA 21-1 and CEA levels had adverse prognostic significance in operated stage I NSCLC patients. Both markers were able to identify a group of patients who were at higher risk of early death despite complete tumor resection. Elevated CYFRA 21-1 and CEA levels were associated with a worse outcome in 21.3% and 13.1% of the patients, respectively. The detection rate for the identification of patients with worse outcome increased when a combination of both markers was used. Thereby, it was possible to identify 32% of the patients where one or both markers were elevated. In order to consider the degree of marker elevation that could reflect tumor burden, we introduced a tumor marker index (TMI) that corresponds to the geometric mean of normalized CYFRA21-1 and CEA levels (marker value /diagnostic cut-off). Thereby, we were able to identify 3 groups of patients at different risk: the first group (22.7%) had a 3-year survival rate of 96.7%, the second group (42.6%) had one of 77.2% and the third group (34.7%) had one of only 55.7%.

The results of our study can only partially be compared with data from the literature since the design of our study differs from those of other reports in several aspects. Firstly, we used exclusively operated stage I patients, secondly,

staging was based on pathological examination (pTNM) and, thirdly, we did not differentiate between the histological subtypes of NSCLC because histology was not a significant prognostic factor in this study.

Despite the differences in the composition of study populations or in the application of tumor markers, a general consensus can be drawn from the results of other investigators. CYFRA 21-1 proved to be the most valuable marker in studies dealing with unstratified NSCLC populations combining any kind of treatment regimen and stages (4, 6). In addition, CYFRA 21-1 was shown to be a significant predictor of survival with regard to the histological subtypes of NSCLC, *i.e.* squamous cell carcinoma (13) and adenocarcinoma (14). In studies using a combination of stages ranging from I-II (13) and I-IIIa (13, 15), CYFRA 21-1 retained its prognostic value. A major problem arises in studies where stratification is based on clinical instead of pathological stages. A substantial stage migration effect between clinical and pathological (postoperative) stages must be taken into account, since a high proportion of patients is known to be misclassified by clinical staging (13, 16, 17).

The prognostic impact of CEA in NSCLC appears less clear. Some authors found only a limited prognostic significance for CEA in adenocarcinoma (14) or squamous cell carcinomas (13), whereas several others could not demonstrate a significant role for this marker at all (6, 15).

In contrast, Suzuki *et al.* (16) and Sawabata *et al.* (17) reported on a considerable prognostic significance of CEA in operated stage I patients. Bearing in mind the above mentioned drawbacks when investigations are based exclusively on clinical staging, the results are very close to our findings with respect to detection and survival rates. It should be mentioned that in these large scale studies a high proportion of adenocarcinomas (>70%) was included. This might explain the prognostic value for CEA, a classical marker for adenocarcinomas. In study populations analyzed by Reinmuth *et al.* (15), Kulpa *et al.* (13) and Nisman *et al.* (6), squamous cell carcinoma was the dominating cell type and only CYFRA 21-1 was of prognostic value.

In conclusion, elevated CYFRA 21-1 and CEA levels were able to identify a group of curatively operated NSCLC patients who were at high risk of early death. Those patients may benefit from more aggressive treatment approaches. In addition, a further group of patients with a 3-year survival rate of 96.7% was identified. These patients probably do not need further treatment.

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