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Preoperative CYFRA 21-1 and CEA as Prognostic Factors in Patients with Stage I Non-Small Cell Lung Cancer

External Validation of a Prognostic Score

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Key Words
CEA · CYFRA 21-1 · External validation · Non-small cell lung cancer · Tumor markers

Abstract
Objective: To validate the prognostic value of preoperative levels of CYFRA 21-1, CEA and the corresponding tumor marker index (TMI) in patients with stage I non-small cell lung cancer (NSCLC). ***Methods:*** Two hundred forty stage I NSCLC patients (80 in pT1 and 160 in pT2; 100 squamous cell carcinomas, 91 adenocarcinomas, 32 large-cell carcinomas, 17 with other histologies; 171 males and 69 females) who had complete resection (R0) between 1986 and 2004 were included in the analysis. CYFRA 21-1 and CEA were measured using the Elecsys system (Roche) and AxSym-System (Abbott), respectively. Univariate analysis was performed using the Kaplan-Meier method to identify potential associations between survival and age, gender, CYFRA 21-1, CEA and TMI. ***Results:*** Overall 3- and 5-year survival rates were 74 and 64%, respectively. Male gender ($p = 0.0009$) and age >70 years ($p = 0.0041$) were associated with a worse prognosis; there were no differences between pT1 and pT2 nor between histological subtypes. Three-year survival was 72% for CYFRA 21-1 levels >3.3 ng/ml versus 75% for levels ≤3.3 ng/ml, 71% for CEA >6.7 ng/ml versus 75% for CEA ≤6.7 ng/ml (both p values >0.05). Corresponding 5-year survival rates were near 64% both for patients with CYFRA 21-1 values above and below the cutoff (3.3 ng/ml), and 49 and 66% for patients with values above and below the CEA cutoff (6.7 ng/ml), respectively (both p values >0.05). Overall survival did not vary in the different TMI risk groups ($p = 0.73$). ***Conclusions:*** In this cohort of early-stage NSCLC patients, male gender and age >70 years were associated with a worse outcome, but elevated levels of CEA and CYFRA 21-1, and TMI risk were not.

Introduction

With over one million deaths in 2002 [1] and over 160,000 in the United States (US) alone in 2005 [2], lung cancer remains one of the leading causes of death worldwide. Resection is still the only therapy recommended to treat stage I non-small cell lung cancer (NSCLC) by the

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International Union against Cancer [3, 4], with only few studies demonstrating a potential benefit of adjuvant platinum-based chemotherapy for stage IB [5, 6]. Therefore, a current challenge in NSCLC treatment is to identify patients that might benefit from more aggressive therapy versus those that may be overtreated. In addition to standard risk factors, in a study by Muley et al. [7] elevated levels of oncological biomarkers (CEA and CYFRA 21-1) were associated with a worse outcome in stage I NSCLC patients. Muley et al. [7] therefore proposed a prognostic score, the tumor marker index (TMI), comprising normalized CEA and CYFRA 21-1 values, and defined three prognostic groups based on the increasing mortality risk according to the TMI score. They concluded that stage I NSCLC patients in the group with the worst prognosis according to the TMI might benefit from further therapy beyond surgery alone. Such a prognostic score is desirable for stage I NSCLC patients since even in patients at this early stage, prognosis is poor, with a 5-year survival rate of approximately 60%. Against this backdrop, the aim of this study was to verify the results reported by Muley et al. [7] and to validate the TMI in an independent cohort of patients.

Patients and Methods

Patients

Preoperative data and clinical follow-up of 240 patients diagnosed with NSCLC in pathological stage I and treated between 1986 and 2004 by complete resection (R0) at the University of Munich Hospital in Grosshadern, Munich, were included in the retrospective study. In addition to pT stage and histology, data from CEA and CYFRA 21-1 levels determined within 30 days prior to surgery were required for inclusion in the study. Patients with known malignancies from other organs were excluded. The diagnosis of lung cancer was confirmed by pathological examination and classified according to the World Health Organization criteria [8]. Postoperative tumor stage was defined by the revised International System for staging of Lung Cancer [9].

Tumor-Associated Antigens

Blood samples were taken within 30 days prior to surgery. CEA was determined with an Abbott AxSYM Analyzer (Abbott Laboratories, Chicago, Ill., USA) using the microparticle enzyme immunoassay. Starting from 1992, CYFRA 21-1 was routinely assessed by an Elecsys kit (Roche Diagnostics, Mannheim, Germany) in all patients with suspected lung cancer. In patients treated before 1992, serum samples stored at –80°C were used for CYFRA 21-1 determination.

TMI

In the study by Muley et al. [7], CYFRA 21-1 and CEA were both assessed by the Cobas Core System of Roche Diagnostics. The TMI was defined by taking the geometric mean of normalized values of CYFRA 21-1 and CEA, where normalization was performed by dividing individual marker values by corresponding diagnostic cutoff points, i.e. 3.3 ng/ml for CYFRA 21-1 and 5.0 ng/ml for CEA:

$$\mathrm{TMI} = \sqrt{\frac{\text{CYFRA 21-1 ng/ml}}{\text{3.3 ng/ml}} \cdot \frac{\text{CEA ng/ml}}{\text{5.0 ng/ml}}}$$

In this study, TMI was calculated accordingly with the exception of using 4.0 ng/ml as the normalizing factor for CEA due to the different assay system; this was not necessary for CYFRA 21-1 since the Elecsys system employs the same diagnostic cutoff point.

Statistical Analysis

Univariate Kaplan-Meier survival curves and rates were calculated, with statistical significance assessed using the log-rank test. To be able to compare our results with those of Muley et al. [7], the cutoff points for age and CYFRA 21-1 were 70 years and 3.3 ng/ml, respectively, and the same three risk groups for TMI were assessed: <0.48 for group A (low risk), 0.48–0.83 for group B (intermediate risk) and >0.83 for group C (high risk). Due to the use of different CEA assays, the cutoff point for CEA was 6.7 ng/ml as opposed to 9.8 ng/ml in the study by Muley et al. [7] in order to achieve the same percentage of patients with CEA values above the cutoff point (13%). In addition, for CYFRA 21-1 and CEA, all values between the 5th and 95th percentiles were evaluated as potential cutoff points using the log-rank test, with statistical significance being assessed at the down-adjusted level of $\alpha = 0.0017$, as recommended for multiple testing [10]. All other statistical tests were performed at the two-sided $\alpha = 0.05$ level of statistical significance. All analyses were performed using the statistical package of SAS (version 9.1, SAS, Cary, N.C., USA).

Results

The characteristics of the 240 study patients and those of the study by Muley et al. [7] are given in table 1. Their study comprised 153 stage I patients, all treated surgically (R0) between 1996 and 1998 at the Clinic for Thoracic Diseases of the University of Heidelberg. Table 1 shows that patient characteristics did not differ between both studies (all $p > 0.05$).

The 3- and 5-year overall survival rates did not significantly differ between our (74 and 64%, respectively), and their study (75 and 58%, respectively; table 2). Median survival of female patients was 13 years, and in males survival was statistically significantly shorter. Age >70 years was also associated with a poorer outcome. There were neither statistically significant differences in survival between pT1 and pT2 stage patients or between histological subtypes nor between adenocarcinomas and squamous cell carcinomas, in agreement with the study by Muley et al. (table 2).

Table 3 lists tumor marker levels in their and our study groups, and in our patients according to pT stage. CYFRA 21-1 levels were similar in both studies, whereas CEA levels were lower in our study due to the different assay system used. Marker levels were higher in pT2 compared to pT1 stage patients.

Table 1. Characteristics of the lung cancer patients from Munich and Heidelberg

Characteristics	Munich (n = 240)	Heidelberg (n = 153[a])	p value[b]
Age, years			
Mean	63.6	64.8	
Range	23–81	32–78	
Male	171 (71.2%)	121 (79.1%)	0.08
Female	69 (28.8%)	32 (20.9%)	
Squamous cell carcinoma	100 (41.7%)	59 (38.6%)	
Adenocarcinoma	91 (37.9%)	75 (49%)	0.07
Large-cell carcinoma	32 (13.3%)	10 (6.5%)	
Other histologies	17 (7.1%)	9 (5.9%)	
pT1 stage	80 (33.3%)	51 (33.3%)	1.00
pT2 stage	160 (66.6%)	102 (66.6%)	
Died during follow-up			
Total	111 (46.3%)	not reported	
Male	88 (36.7%)	not reported	
Female	23 (17%)	not reported	

[a] Extracted from Muley et al. [7].
[b] χ^2 test for differences between the two studies.

Three- and 5-year survival rates for preoperative CYFRA 21-1 and CEA levels of patients grouped according to the cutoff points are described in table 4. Based on these cutoff points, neither CYFRA 21-1 nor CEA was associated with survival (p = 0.67 for CYFRA 21-1 and p = 0.09 for CEA). In contrast, a significant association between CYFRA 21-1 and 3-year survival was reported in the study by Muley et al. (p = 0.015), with a markedly lower 3-year survival rate for patients with CYFRA 21-1 levels above the cutoff point compared with this study (table 4).

TMI was calculated for all of our patients according to the risk groups described by Muley et al. [7]. In their study, the low-, intermediate- and high-risk groups com-

Table 3. Distribution of biomarkers (ng/ml)

	Munich (n = 240)		Heidelberg (n = 153)	
	n	median (5th–95th percentiles)	n	median (5th–95th percentiles)
CYFRA 21-1				
All	240	1.8 (0.9–7.5)	141	1.8 (0.9–8.0)
pT1	80	1.2 (0.5–3.2)		not reported
pT2	160	2.6 (0.5–13.2)		not reported
CEA				
All	240	2.7 (0.9–13.8)	153	3.5 (1.1–21.0)
pT1	80	2.6 (0.9–8.6)		not reported
pT2	160	2.8 (0.9–25.5)		not reported

Table 2. Univariate analysis of survival

	Median survival months	Munich			Heidelberg p value log-rank test
		5-year survival, %	3-year survival, %	p value log-rank test	
Overall	110	63.9	74.4		
Sex				<0.001	0.029
Male	86	58.0	70.4		
Female	156	76.7	83.5		
Age				0.004	0.026
<70 years	125	69.6	77.8		
>70 years	58	47.6	64.1		
Histological type				0.16	0.18
Squamous cell carcinoma	105	64.5	72.0		
Adenocarcinoma	125	63.2	76.7		
Large cell carcinoma	61	55.6	65.4		
Other histology	123	77.9	92.9		
Pathological stage				0.146	0.14
pT1	125	71.5	81.1		
pT2	96	59.9	70.9		

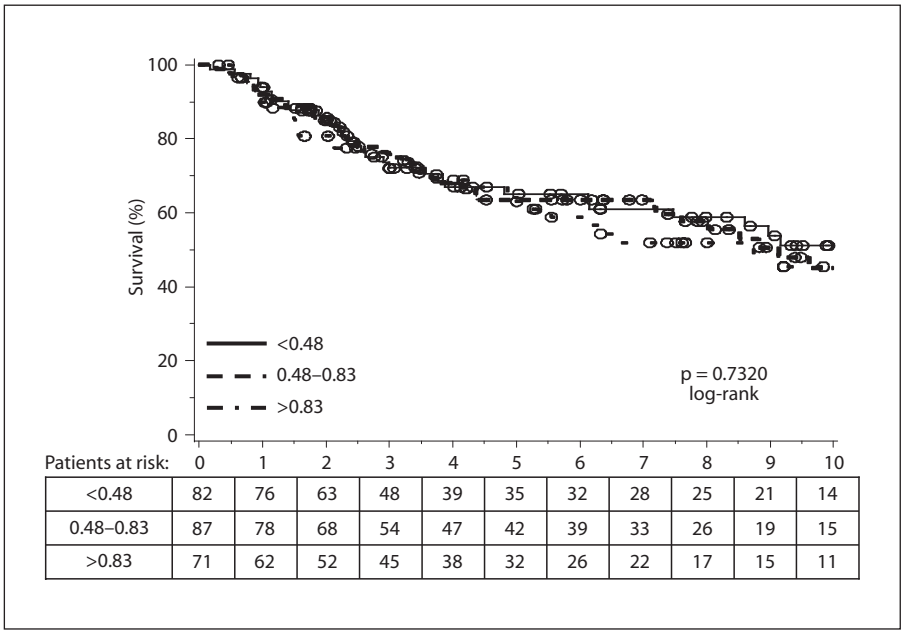


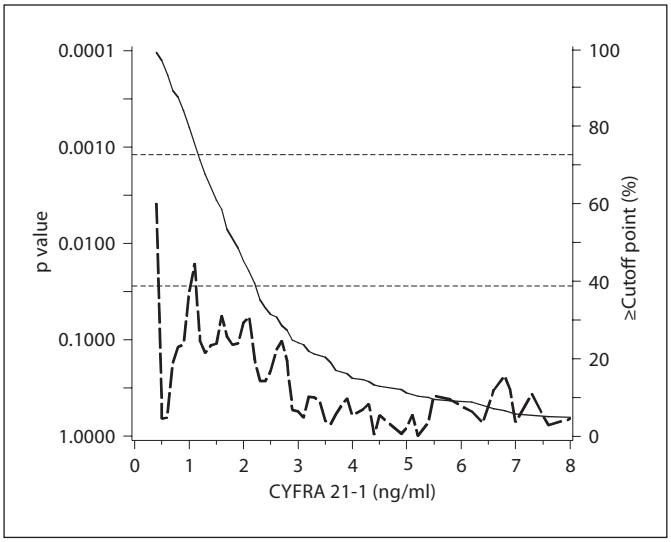
Fig. 1. Survival curves by TMI.

Table 4. Univariate analysis of survival and biomarkers (ng/ml)

	Median survival months	Munich			Heidelberg	
		5-year survival, %	3-year survival, %	p value	3-year survival, %	p value
CYFRA 21-1				0.67		0.015
>Cutoff point	102	64	72.3		60.2	
≤Cutoff point	110	63.8	74.9		78.4	
CEA				0.09		0.014
>Cutoff point	59	49	70.8[a]		41.6[b]	
≤Cutoff point	110	66.1	74.9[a]		79.2[b]	
TMI				0.73		
High-risk group	105	63.1	75.9		55.7	0.0008 vs. low
Intermediate-risk group	110	63.5	75.0		77.2	0.077 vs. high
Low-risk group	122	65.1	72.1		96.7	0.017 vs. low

Cutoff point for CYFRA 21-1: 3.3 ng/ml.
[a] Cutoff point: 6.7 ng/ml. [b] Cutoff point: 9.8 ng/ml.

prised 22.7, 42.6 and 34.7% of the patients, respectively, showing statistically significant differences in survival rates (table 4). Neither CEA or CYFRA 21-1 levels nor TMI were statistically significant in this study (p = 0.7320; fig. 1; table 4). Therefore, alternative cutoff points for CYFRA 21-1 and CEA were investigated to independently assess the prognostic information of these biomarkers. For CYFRA 21-1 none of the cutoff points indicated prognostic value at the adjusted 0.0017 level of statistical significance (fig. 2). This was also true when only squamous cell carcinomas and adenocarcinomas were analyzed. CEA cutoff points ≥9 ng/ml indicated statistical significance at the adjusted α level (p = 0.0015). However, <10% of the patients had CEA levels above this cutoff point (fig. 3). For CEA, no other significant cutoff points were found restricting the patient group to adenocarcinomas or squamous cell carcinomas.



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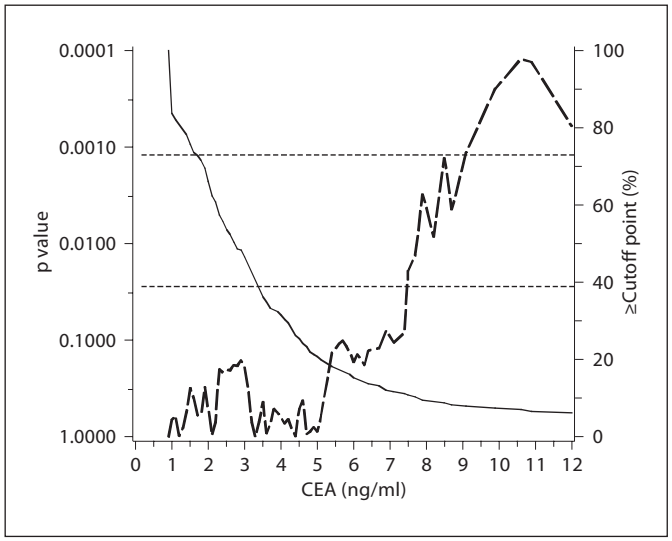

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Fig. 2, 3. Evaluation of alternative cutoff points of CYFRA 21-1 (**2**) and CEA (**3**) for associations with mortality; solid curve: percentage of patients with values above the corresponding cutoff points; dashed curve: p values from log-rank test for each cutoff point; lower horizontal dashed line: p value of 0.05; upper horizontal line: adjusted p value [10].

Discussion

Survival rates for patients suffering from NSCLC are dismal, even for early-stage disease. While surgery is currently the only treatment recommended for NSCLC, evidence accumulates that some patients might benefit from adjuvant therapy [11, 12]. Prognostic factors such as the TMI might help to separate patients likely to benefit from adjuvant therapy from those who have a good prognosis postoperatively.

Overall survival rates in this study (74% for 3 years and 64% for 5 years) were similar to those reported by Muley et al. [7] and others [13–16]. Two studies reported a survival rate of approximately 80% at 5 years [17, 18], but these studies had a higher percentage of female patients and in one of them [18], patient follow-up was only 36 months versus 110 months in our study. The previously established prognostic factors age and sex [7, 13, 17, 18] were confirmed in this investigation, whereas tumor size or histology were not associated with survival, in accord with the results of Muley et al. [7]. However, the results of this study neither confirmed the association of TMI to survival nor the prognostic significance of CYFRA 21-1. In studies including patients with NSCLC at all stages [19–22] or those restricted to patients with squamous cell carcinomas of the lung [15, 23, 24], high CYFRA 21-1 levels have been associated with a poorer outcome independent of histology. Most of them used clinical staging to diagnose early-stage NSCLC, leading to bias due to potential misstaging [17, 25–27]. To our knowledge, only two studies on CYFRA 21-1 and CEA levels have been conducted exclusively in pathological stage I patients [7, 18]. Muley et al. [7] demonstrated independent significant prognostic significance for CYFRA 21-1, and Matsuoka et al. [18] revealed CYFRA 21-1 as a significant prognosticator for overall survival but not time to progression.

Although CEA is one of the earliest available biomarkers, its role as a prognosticator for survival in NSCLC remains controversial. The prognostic value of CEA was demonstrated in several studies [7, 25, 26, 28] in contrast to others [20, 29, 30], which may be due to the heterogeneous histological subtypes. Investigations including a high percentage of patients with squamous cell carcinoma [20, 24] found a statistically significant association between elevated preoperative CEA levels and poorer survival, whereas the study by Nisman et al. [23] showed statistical significance only in patients with adenocarcinoma. In our study, statistically significant associations between CEA and survival could only be demonstrated for preoperative CEA levels >9 ng/ml or on a continuous scale. The detection of prognostic significance depends on the cutoff point selected. Thus, more detailed examinations covering a range of CEA cutoff points and scales may aid in the detection of associations. Findings from these investigations require external validation since data

mining of a particular dataset is used to identify the optimal cutoff point, with a high probability of not being exactly replicated in other studies. One reason for the differences in the prognostic values of CYFRA and CEA found across studies could be the incomparability of immunoassays across different assay systems, which is particularly striking when selected cutoff points are used. For this reason, alternative cutoff points for CEA and CYFRA 21-1 to those recommended by Muley et al. [7] were investigated in this study. Another limitation is the heterogeneity of the patient cohorts. These inconsistencies should be resolved by further external validations, preferably across multiple centers.

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