J Lab Med 2008;32(5):361–371 © 2008 by Walter de Gruyter • Berlin • New York. DOI 10.1515/JLM.2008.050

Onkologie Redaktion: P. Stieber

CEA, CYFRA 21-1, NSE, and ProGRP in the diagnosis of lung cancer: a multivariate approach

CEA, CYFRA 21-1, NSE und ProGRP in der Diagnostik des Lungenkarzinoms: eine multivariate Analyse

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Abstract

We retrospectively studied the single and combined diagnostic value of carcinoembryonic antigen (CEA), cytokeratin fragment 19 (CYFRA 21-1), neuron specific enolase (NSE) and pro-gastrin-releasing peptide (ProGRP), which were routinely analysed in patients with lung tumours of unknown origin at the time of admission to hospital. Inclusion criteria were the determination of CEA (AxSYM/Abbott), CYFRA 21-1 (ElecSys/Roche) and NSE (Kryptor/Brahms). We examined 1747 patients, where 1325 suffered from lung cancer (LC; small cell lung cancer, SCLC: n=194; non-small cell lung cancer, NSCLC: n=1015; others: n=116), 318 from benign lung diseases and 104 from lung metastases due to another primary malignancy. As ProGRP (ELISA ALSI/IBL) became available only recently, there are less data points of this marker. In total, 99.8% of LC patients released at least one of the four biomarkers (defined as values exceeding the median of healthy controls), and for the discrimination between benign disease (BD) and malignant lung disease each marker reached 100% tumour specificity at high levels (CEA: 20 ng/mL; CYFRA 21–1: 40 ng/mL; NSE: 45 ng/mL; ProGRP: 250 pg/mL). At a specificity of >99%, ProGRP reached the highest diagnostic efficacy for SCLC with 57% true positive results, CEA had the highest capacity (17%) to detect malignant lung tumours in general and adenocarcinomas of the lung with 29%. CYFRA 21-1 was dominant for squamous cell carcinomas (12%). Combining the four markers leads with the prerequisite of high specificity (>99%) to 50% true positives for malignant lung tumours, 44% for NSCLC, 36% for squamous cell carcinomas, 53% for adenocarcinomas, and 78% for SCLC, respectively. In cases of lung tumours of unknown origin, the combined use of CEA, CYFRA 21-1, NSE and ProGRP is useful for the differentiation between benign and primary or secondary malignant disease and suggests the assignment to histological subtypes.

Keywords: carcinoembryonic antigen (CEA); cytokeratin fragment 19 (CYFRA 21-1); logistic regression; lung cancer; multivariate analysis; neuron specific enolase (NSE); pro-gastrin-releasing peptide (ProGRP).

Zusammenfassung

Im Rahmen einer retrospektiven Analyse wurde die diagnostische Wertigkeit von CEA, CYFRA 21-1, NSE und ProGRP untersucht – einzeln und in Kombination – die im Rahmen der stationären Aufnahme zur Abklärung eines Lungentumors routinemässig angefordert wurden.

Einschlusskriterium war das Vorhandensein von CEA (AxSYM/Abbott), CYFRA 21-1 (ElecSys/Roche) und NSE (Kryptor/Brahms) bei jedem Patienten. Wir untersuchten insgesamt die Proben von 1747 PatientInnen, darunter 1325 PatientInnen mit einem Lungenkarzinom (=LC, kleinzelliges Lungenkarzinom=small cell lung cancer SCLC: n=194, nicht kleinzelliges Lungenkarzinom=non small cell lung cancer NSCLC: n=1015, andere Subformen: n=116), sowie 318 Proben von PatientInnen mit benignen Lungenerkrankungen und Proben von 104 PatientInnen mit Lungenmetastasen aufgrund eines anderen Primärtumors. Da ProGRP (ELISA ALSI/IBL) erst seit einigen Jahren für Routinemessungen verfügbar ist, ist die Anzahl der ProGRP messungen geringer verglichen mit den 3 anderen Biomarkern.

99.8% der LC PatientInnen setzten mindestens einen der 4 Biomarker gesteigert frei (>Median gesunder Kontrollen), bei hohen Konzentrationen erreichte jeder der 4 Marker zur Unterscheidung zwischen benigner (BD) und maligner Lungenerkrankung eine 100%ige Tumorspezifität (CEA: 20 ng/mL; CYFRA 21-1: 40 ng/mL; NSE:

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45 ng/mL; ProGRP: 250 pg/mL). Bei einer Spezifität >99% erreichte ProGRP mit 57% richtig positiven Testergebnissen die höchste diagnostische Effizienz für SCLC, CEA hatte mit 17% die höchste Fähigkeit zur Entdeckung eines malignen Lungenrundherdes im allgemeinen sowie mit 29% zur Entdeckung von Adenokarzinomen der Lunge. CYFRA 21-1 dominierte in der Entdeckung von Plattenepithelkarzinomen (12%). Basierend auf der Forderung einer 99%igen Spezifität führte die Kombination aller 4 Marker zur Entdeckung von 50% der malignen Lungenrundherde, von 44% für das NSCLC, 36% für Plattenepithelkarzinome, 53% für Adenokarzinome und 78% für kleinzellige Karzinome.

Zusammenfassend kann festgestellt werden, dass im Falle von unklaren Lungentumoren die kombiniertte Bestimmung von CEA, CYFRA 21-1, NSE und ProGRP sowohl für die Diffeenzierung zwischen benignen und malignen Lungenrundherden hilfreich ist, als auch die Zuordnung zu histologischen Subtypen von malignen Lungentumoren ermöglicht.

Schlüsselwörter: Cytokeratin 19-Fragment (CYFRA 21-1); Karzinoembryonales Antigen (CEA); Logistiche Regression; Lungenkarzinom; Multivariate Analyse; Neuronspezifische Enolase (NSE); ProGastrin Releasing Peptid (ProGRP).

Introduction

Lung cancer is the most frequent cancer in the world, both in terms of incidence (1.2 million new cases or 12.3% of the world total) and mortality (1.1 million deaths or 17.8% of the total). For males, it is by far the most common cancer worldwide (incidence rate 34.9 per 100,000). In females, incidence rates are lower (11.1 per 100,000). Trends in lung cancer incidence and mortality are related to the smoking epidemic in different countries. In men, several populations have passed the peak of tobacco consumption, and incidence and mortality decrease slowly in the USA and several European countries. In contrast, most western countries show a rising trend in incidence and mortality in women [1–3].

Histopathologically, lung cancer is divided into two types: non-small cell and small cell lung cancer (NSCLC and SCLC). The former consists of several subtypes, predominantly adenocarcinoma, squamous cell carcinoma and large cell carcinoma. SCLC is a more aggressive carcinoma with neuroendocrine capacities and accounts for 15% to 25% of lung cancer patients. Many lung cancers constitute histologically mixed tumour types consisting of non-small cell and small cell components [4–6].

Patients with lung cancer, particularly in the early stages, often do not exhibit specific signs and symptoms. Dyspnoea, cough, thoracic pain are considered non-specific early signs, haemoptysis may already indicate advanced stages of lung cancer. Relapsing infectious diseases of the respiratory system in combination with a smoking history might be an indication for further examination. The diagnostic work-up for lung cancer includes medical history and physical examination, clinical laboratory testing, chest radiography, computed tomography or magnetic resonance imaging of the chest, abdomen and the brain, bronchoscopy, biopsy, bone scan, preoperative pulmonary function studies, and eventually positron emission tomography, bone marrow biopsy and thoracentesis [5, 6].

Histological differentiation and lung cancer staging is mandatory for therapeutic stratification. For patients with NSCLC, particularly for those with early stages (I–IIIA), surgery is the mainstay of treatment. The additional application of adjuvant radio- or chemotherapy provides only minimal benefit in certain subgroups of patients which are difficult to define by current techniques. The use of neoadjuvant systemic therapy to provoke tumour shrinkage and early eradication of systemic micrometastases is still a matter of debate [4, 5]. Five-year survival rates depend strongly on tumour stage with 60% to 70% for stage I, 40% to 50% for stage II, and 15% to 30% for stage IIIA [5]. Currently, virtually no patient with non-resectable non-small lung cancer in advanced stage (IIIB and IV) will be cured. Median survival for stage IV patients has been stable for years at 8–10 months. Although response rates for chemo- and radiotherapy are low, several studies have demonstrated moderate beneficial effects concerning survival, time to disease progression, and quality of life, as compared with best supported care (reviewed in [4]). Combined radio-chemotherapy is suggested particularly for patients with stage IIIB disease [4, 7]. Patients with stage IV disease are mostly treated by chemotherapeutic regimens, except for those with localised single metastases who may be eligible for surgical resection [4, 5, 7].

Small cell lung cancer is characterised by a rapid doubling time and its propensity for early metastases. In contrast to NSCLC, staging is only related to the extent of disease that alters prognosis and treatment decisions. In clinical practice, only two stages of SCLC are distinguished: limited stage disease (LD) with the tumour confined to one hemithorax only versus extensive stage disease (ED) with metastases in the opposite chest or at distant sites. In total, 20% to 25% of patients will have limited disease, which can be treated with curative intention. However, the 5-year survival rates are still low (15% to 25%, compared to extensive disease with <5%). In these patients, a multimodal approach of chemo- and radiotherapy is recommended followed by prophylactic cranial irradiation to prevent cerebral metastases.

Lung cancer belongs to a set of solid tumours releasing oncological biomarkers, including carcinoembryonic antigen (CEA) [8], cytokeratin fragment 19 (CYFRA 21-1) [9–14], neuron specific enolase (NSE) [15] and pro-gastrin-releasing factor (ProGRP) [16–20] at an early stage of disease into blood. Selected biomarkers, notably NSE and ProGRP, have the diagnostic advantage to be released only by one of the various histological subtypes

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of lung cancer, namely SCLC. Therefore, they might be valuable for the differential diagnosis particularly when a biopsy cannot be performed for topographic or clinical reasons. The clinical value of the information provided by tumour markers in these cases is crucial, because the therapeutic approach strongly depends on the classification as SCLC versus NSCLC.

Furthermore, the decrease of tumour markers after surgery may indicate the completeness of the removal of tumour tissue or the presence of micrometastatic residues. Intensity of the follow-up schedule can be supported by tumour marker kinetics. Moreover, the pre- and post-therapeutic concentrations provide additional prognostic information to the clinical staging that will be helpful for further therapeutic stratification, particularly if histologically mixed tumour types are present or several adjuvant therapies are available.

The aim of the present investigation was to answer the following questions: How efficient is the combined use of the biomarkers CEA, CYFRA 21-1, NSE and ProGRP for the discrimination between benign and malignant lung diseases? Are these markers able to discriminate between primary lung cancer and lung metastases due to another primary malignancy? Do these markers support histological classification?

Patients and methods

Study design and patients

This retrospective study includes 1747 patients who were referred to the Department of Thoracic Surgery during the years 1986–2003, either to clarify the diagnosis of lung tumours of unknown origin or to operate on already known malignant lung tumours.

Inclusion criteria were pre-therapeutic values of CEA, CYFRA 21-1 and NSE. ProGRP became available only 3 years ago resulting in less data points.

Of the 1747 patients, 1325 suffered from primary lung cancer, 318 had benign lung diseases and 104 patients had lung metastases due to another primary malignancy. The characteristics of the patients are provided in Table 1. Patients with NSCLC were classified according to UICC (Union Internationale Contre le Cancer) staging, patients suffering from SCLC were grouped according to limited versus extensive disease.

Gastrointestinal cancer was the most frequent primary malignancy (n = 37) in the lung metastases group (n = 104), followed by urogenital cancer (n = 25), breast cancer (n = 16), lymphoma/sarcoma (n = 4), cancer of endocrine origin (n = 5), cancer of the ear, nose and throat (n = 5), and of unknown origin (n = 12).

The group of 318 patients with benign lung diseases included patients with acute infections (tuberculosis, pneumonia, pleural empyema, pleuritis and bronchitis), acute lung disease (pneumothorax, pleural effusion, haemoptysis and atelectasis), chronic lung disease

Table 1 Characteristics of the patients.

	Lung cancer	All	Squamous cell	Adeno cell	Large cell	Non-small cell carcinoma without further classification	NSCLC	SCLC	Unknown histology and others	Lung metastases	Benign lung disease
Patients, n	1325	449	361	138	67		1015	194	116	104	318
(male/female)	(1019/306)						(802/213)	(141/53)	(76/40)	(52/52)	(220/98)
Median age	63.2						63.4	62.5		63.3	57.3
(range), years	(14–87)						(14–87)	(35–86)		(30–93)	(16–91.5)
UICC stage for NSCLC											
I		100	74	23	6		203	11	28		
II		75	29	10	4		118	9	6		
III		81	67	19	6		173	20	5		
IV		42	87	27	21		177	61	18		
Stage missing for SCLC	67										
Stage LD for SCLC	65										
Stage ED for SCLC	62										

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(sarcoidosis, aspergilloma, bronchogenic cyst, lung cyst, hamartoma, chronic pneumonia, pleural fibrosis, COPD (chronic obstructive pulmonary disease), chronic bronchitis, bronchial asthma, pulmonary emphysema, bronchiectasis, pneumoconiosis, interstitial lung disease, pulmonary fibrosis) and benign lung tumours.

Assays

All tumour markers were assessed using commercial kits in the Institute of Clinical Chemistry of the University Hospital of Großhadern, Munich. For CEA, the AxSYM test system employing the principle of microparticle enzyme immunoassay (MEIA) from Abbott Diagnostics (Chicago, Illinois, USA) was used. There were methodical changes during the time course of data collection for the tumour markers CYFRA 21-1 and NSE. CYFRA 21-1 was initially measured by the enzyme immunoassay (EIA) on the ES 600 system of Boehringer Mannheim (Mannheim, Germany) and later by the Elecsys EIA by Roche Diagnostics (Mannhein, Germany). Samples of 114 patients were analysed for CYFRA 21-1 using both tests. For the purpose of this specific study, we performed a regression analysis (Passing-Bablok algorithm), including CYFRA 21-1 values <15 ng/mL. This range of values was selected, as low to moderate concentrations of oncological biomarkers are of special importance for diagnostic purposes. CYFRA 21-1 values of the ES 600 test were converted by the equation: CYFRA 21-1 (Elecsys) = CYFRA 21-1 (ES 600)–0.3, if the CYFRA 21-1 (ES 600) value was >0.4 ng/mL to avoid negative concentrations.

For NSE analyses, three different methods were used: Pharmacia Diagnostics Sweden (RIA) (Uppsala, Sweden), Roche Diagnostics on the Cobas Core (EIA), and KRYPTOR TRACE by Brahms Diagnostica (Berlin, Germany). For 183 patients with parallel determinations using the tests by Pharmacia and Roche and NSE values <20 ng/mL, the Passing-Bablok regression equation was: NSE (Cobas) = 1.52 × NSE (Pharmacia)–1.826. Furthermore, we calculated the Passing-Bablok regression for 189 patients measured with Cobas Core and KRYPTOR TRACE principle. The result was NSE (KRYPTOR) = 0.769 × NSE (Cobas) + 1.008. It should be noted that the former calculations would not apply for routine purposes.

For ProGRP, the ELISA principle by ALSI (Saitama, Japan) (IBL, Hamburg, Germany) was used.

Statistical analysis

Tumour marker values are tabulated as median, quartiles and range for the different groups of benign lung diseases, metastases due to another primary malignancy, and lung cancer as well as its subdivision into SCLC and NSCLC. In addition, box plots (median, 5th and 95th percentile, extreme values as dots) for the four markers and the major diagnostic groups of benign lung diseases, lung metastases and primary lung cancer were performed using a logarithmic scale.

To analyse associations between tumour marker values and T, N and M stage for the NSCLC group and between tumour marker values and the stage of limited disease and extensive disease for the SCLC group, respectively, a trend test on ranks of values was performed. Correlations between different tumour markers were calculated using the Spearman rank coefficient.

To compare the serum tumour markers CEA, CYFRA 21-1, NSE and ProGRP for their ability to discriminate between benign and malignant lung diseases, specificity and sensitivity was calculated using the receiver operating characteristic (ROC) in addition to the area under the curve (AUC), which was calculated as the Wilcoxon rank sum test statistic and a test of the null hypothesis that the AUC is 50% versus the alternative that it exceeds 50%. Sensitivities for the single markers and their combined use were calculated at a specificity of $>99\%$ for benign lung diseases.

For a logistic regression model, biomarkers were included as continuous variables on a natural logarithmic scale.

The coefficients of the logistic regression model allow for the calculation of a score. Scores presented in this study are calculated using leave-one-out validation, meaning that for each patient those coefficients were used which are based on a model which was calculated without this patient.

Results

Tumour marker values

Figure 1 demonstrates box plots for the four tumour markers in benign diseases, lung metastases and primary lung cancer. As compared to benign lung diseases, values of all four tumour markers were significantly higher for patients with primary lung cancer ($p=0.0000$ for CEA, CYFRA21-1 and NSE; $p=0.004$ for ProGRP), and CEA and CYFRA21-1 also for patients with lung metastases due to another primary malignancy (CEA: $p=0.001$; CYFRA 21-1: $p=0.0001$). This difference was less pronounced for NSE ($p=0.04$) and did not reach significance for ProGRP ($p=0.7$). Differences between patients with primary lung cancers and patients with lung metastases were only significant for CEA ($p=0.0003$) and CYFRA 21-1 ($p=0.005$).

Tumour marker values (median and 95% percentiles) for histological subgroups of lung cancer patients are provided in Table 2. As compared to patients with NSCLC, ProGRP values were markedly elevated in patients with SCLC, and to a lower extent also NSE levels. No difference was found for CEA, whereas CYFRA21-1 values were slightly lower in patients with SCLC.

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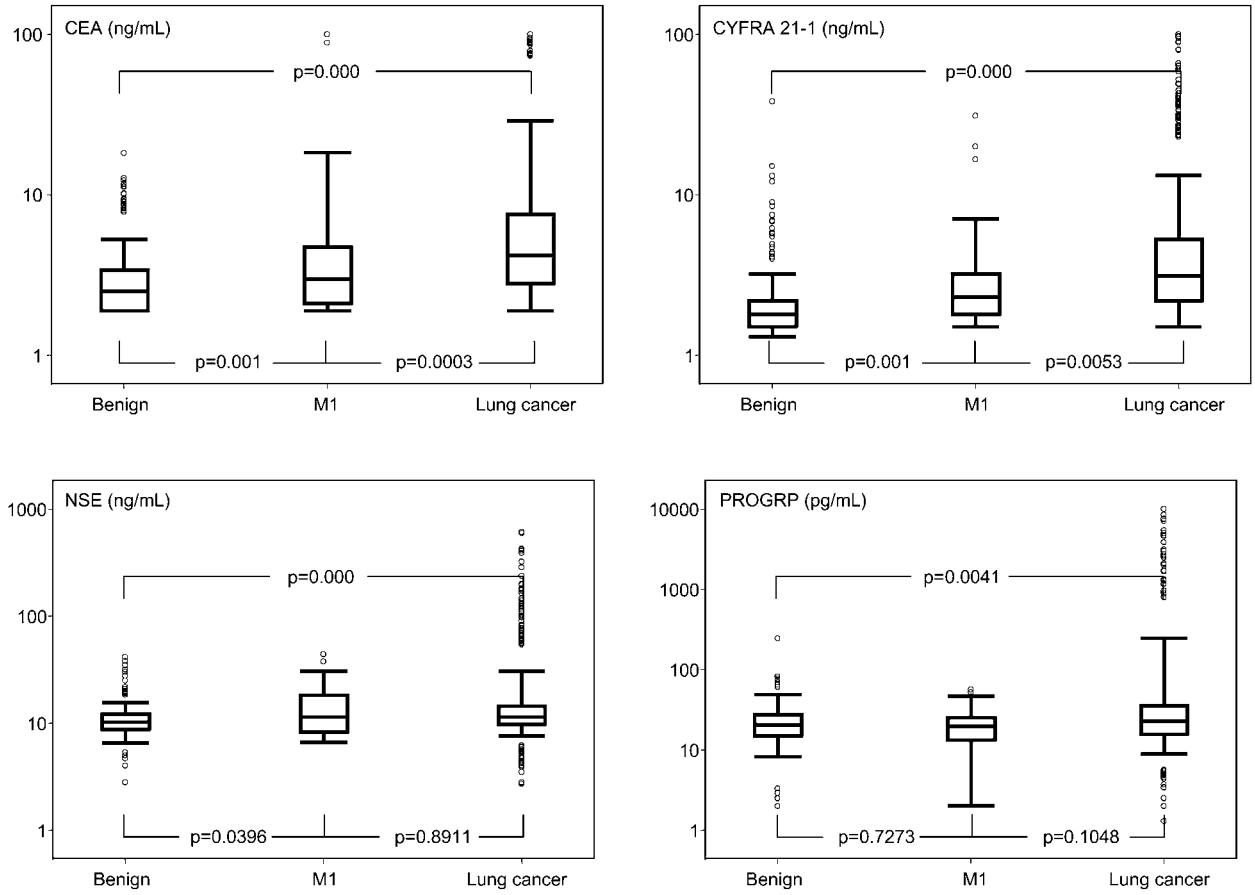

Figure 1 Distribution of CEA, CYFRA 21-1, NSE and ProGRP (medians, quartiles, 5th and 95th percentiles, highest values in dots) in benign lung diseases (n = 318), lung metastases (n = 104) and primary lung cancer (n = 1325).

In the NSCLC group, the highest CEA values were reached in adenocarcinomas, followed by large cell carcinomas, whereas in squamous cell carcinomas levels were significantly lower. In contrast, CYFRA 21-1 values were highest in squamous cell carcinomas, followed by large cell carcinomas and lowest in adenocarcinomas. The highest values of NSE were found for large cell carcinomas. No differences between histological subtypes of NSCLC were obtained for ProGRP.

We classified patients with NSCLC according to UICC staging and patients with SCLC into extensive and limited disease (Table 3). Significantly increased marker concentrations were observed with more advanced tumour stages in patients with NSCLC for the tumour markers CEA ($p < 0.0001$), CYFRA 21-1 ($p < 0.0001$) and NSE ($p = 0.001$). Patients suffering from SCLC with extensive disease showed significantly higher CEA ($p < 0.05$), CYFRA 21-1 ($p = 0.0002$) and NSE ($p = 0.0000$) concentrations compared to limited disease. With regard to ProGRP, there was no significant correlation with tumour stage for SCLC or for NSCLC.

Each marker reached 100% tumour specificity at high levels. For CEA, the value is 20 ng/mL, for CYFRA 21-1 it is 40 ng/mL, for NSE it is 45 ng/mL, and for ProGRP it is 250 pg/mL, respectively. Considering 100% specificity, CEA reached the best diagnostic sensitivity for malignancy (lung metastases and primary lung cancer) with 12.5%, followed by ProGRP with 9%, NSE with 6% and CYFRA 21-1 with only 2%. Furthermore, we looked at 99% specificity of each tumour marker for benign lung diseases and calculated at this value the sensitivity of the markers for the differential diagnosis benign lung diseases, lung metastases and lung cancer, and histological subtypes. Table 3 demonstrates that all four markers are able to discriminate between benign and malignant lung diseases, whereas CEA is the most efficient with 17% compared to 0.3% in the benign lung diseases group. Except for ProGRP, which reached a sensitivity of 13% in the lung cancer group in comparison to 0% in the lung metastases group, no marker could differentiate between lung cancer and lung metastases at the high cut-off level. Regarding the NSCLC and SCLC groups, the markers NSE (40% SCLC versus 3% NSCLC) and ProGRP (57% SCLC versus 2% NSCLC) are able to discriminate between these two groups. In the NSCLC subgroups, the highest sensitivity of CEA (29%) is reached in adenocar-

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Table 2 Distribution of CEA, CYFRA 21-1, NSE and ProGRP serum concentrations, subdivided according to the histological type of lung cancer.

	CEA (ng/mL)				CYFRA 21-1 (ng/mL)				NSE (ng/mL)				ProGRP (pg/mL)			
	n	Med	P 95	p	n	Med	P 95	p	n	Med	P 95	p	n	Med	P 95	p
NSCLC	1015	3.5	72.6		1015	2.5	25.5		1015	10.2	24.6		439	20.6	54.6	
Squamous cell	449	3.0	23.2	[a, b]	449	3.0	29.0	[b]	449	10.0	20.5	[a]	175	22.5	51.6	
Adeno cell	361	4.6	135.0	[a]	361	1.8	16.0	[a]	361	10.0	20.5	[a]	157	19.9	51.8	
Large cell	138	3.9	155.0		138	2.9	23.7		138	11.1	40.2		57	18.4	66.0	
SCLC	194	3.5	88.0		194	1.8	14.3	[c]	194	25.4	195.6	[c]	109	185.0	5457.0	[c]
All lung cancer patients	1325	3.4	72.9		1325	2.3	22.8		1325	10.6	54.2		612	22.1	801.0	

[a]Significantly different from large cell carcinoma. [b]Significantly different from adeno cell carcinoma. [c]Significantly different from NSCLC. Med: median of the values; P 95: 95th percentile.

Table 3 Sensitivity (%) of CEA, CYFRA 21-1, NSE and ProGRP at a specificity of >99% for benign lung diseases.

Patients	Benign lung disease	Malignant lung disease	Lung metastases	Lung cancer	All	NSCLC	Squamous cell	Adeno cell	Large cell	Non-small cell carcinoma without further classification	SCLC	Unknown histology and other
Marker												
CEA												
≥12 ng/mL	0.3	17	16		18	18	9	29	20	17	16	17
CYFRA 21-1												
≥15 ng/mL	0.3	8	7		8	9	12	5	8	14	5	3
NSE												
≥35 ng/mL	0.6	8	5		8	3	1	2	6	8	40	4
ProGRP												
≥85 pg/mL	0.6	12	0		13	2	0.6	1	4	8	57	9
At least 1 marker												
≥99% specificity	3	50	56		50	44	36	53	51	39	78	38

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Table 4 Distribution of the values of the tumour markers CEA, CYFRA 21-1, NSE and ProGRP according to UICC stage.

Stage of lung cancer	CEA, ng/mL			CYFRA 21-1, ng/mL			NSE, ng/mL			ProGRP, pg/mL		
	n	Median	P 95	n	Median	P 95	n	Median	P 95	n	Median	P 95
NSCLC												
UICC I	203	3.0	20.0	203	1.7	10.1	203	9.6	18.1	59	19.0	52.6
UICC II	118	2.85	23.8	118	2.35	21.7	118	10.0	27.7	35	22.5	64.5
UICC III	173	4.4	109.0	173	2.7	20.6	173	9.9	30.2	54	19.3	51.4
UICC IV	177	6.1	107.0	177	3.4	40.0	177	10.9	28.7	84	21.0	81.9
p	<0.0001			<0.0001			0.0012			0.8372		
SCLC												
LD	62	3.2	14.7	62	1.4	7.9	62	12.9	79.4	27	70.4	3001.0
ED	65	4.0	197.0	65	2.6	30.5	65	51.1	420.0	34	423.5	8462.0
p	0.0447			0.0002			0.0000			0.0753		

cinoma, whereas CYFRA 21-1 (12%) is dominant in the squamous cell carcinoma group. The markers NSE and ProGRP are not able to differentiate the NSCLC subgroups.

We also examined the sensitivities for the same groups if at least one tumour marker reached 99% specificity. For this observation, the sensitivity for SCLC is the highest with 78%, whereas the other groups reached a sensitivity of approximately 50%.

Multivariate discrimination between benign and malignant lung diseases

To quantify the diagnostic capacity of the different tumour markers, we used a logistic regression model including age and all four tumour markers without predestinated cut-off values (Table 5). Only CEA, CYFRA 21-1 and NSE, as well as age provided significant diagnostic information. The additional diagnostic information of ProGRP was non-significant, thus it was excluded from the final analysis.

Regarding the influence of age on the probability of malignancy, a quadratic term was required. The highest probability for malignancy was between the ages of 55 to 70 years.

From the coefficients of the model, the following score can be calculated:

Score (malignancy) $= 0.2676 \times \text{Age} - 0.00208 \times \text{Age}^2 + 0.9932 \times \log(\text{CEA}) + 1.3774 \times \log(\text{CYFRA 21-1}) + 0.7453 \times \log(\text{NSE}) - 11.3808$. We calculated this score, however, using leave-one-out validated coefficients.

Figure 2 shows ROC curves of the score in comparison to single tumour markers. Of these, CYFRA 21-1 reached the best AUC (0.79), followed by CEA (0.72); whereas, the two markers NSE (0.59) and ProGRP (0.57) had less diagnostic relevance. The AUC of the score (0.839) of the combined use of the three tumour markers CYFRA 21-1, CEA and NSE, as well as the age is superior to the AUCs of the single markers.

As mentioned above, values of ProGRP were not available for all patients and therefore the ROC curve of ProGRP is not directly comparable to the ROC curves of the other tumour markers. Using only patients with all four markers available (benign lung diseases: n = 171, malignant lung diseases: n = 632), the results are comparable with the results using the whole sample (CYFRA 21-1: AUC = 0.76, CEA: AUC = 0.73, NSE: AUC = 0.65, ProGRP: AUC = 0.57).

Furthermore, the 1669 patients of the multivariate analysis were subdivided into five equal groups and the amount of benign lung diseases and the amount of malignant lung diseases were counted for each subgroup. The proportions of benign lung diseases and of malignant lung diseases are illustrated in Figure 3. It is evident that the probability of benign lung disease declines continuously, whereas the probability of malignant lung disease increases with higher score levels.

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Table 5 Logistic regression for the discrimination between benign lung disease and malignant lung disease (Ln = natural logarithm).

Variable	Coefficient	Standard error	p	Odds ratio	95% Confidence limits
Age	0.2676	0.0491	<0.0001	1.307	1.187–1.439
Age^2	–0.00208	0.000416	<0.0001	0.998	0.997–0.999
Ln CEA	0.9932	0.1320	<0.0001	2.700	2.084–3.497
Ln CYFRA 21-1	1.3774	0.1610	<0.0001	3.964	2.892–5.436
Ln NSE	0.7453	0.1802	<0.0001	2.107	1.480–3.000
Constant	–11.3808	1.4946	<0.0001		

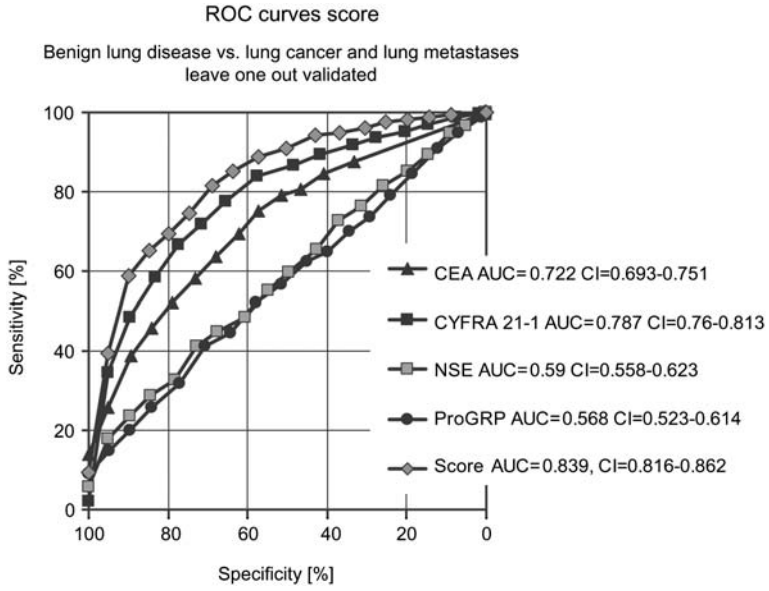

Figure 2 ROC curves and AUC of CEA, CYFRA 21-1, NSE and ProGRP and the established score for benign lung diseases and malignant lung diseases.

Discrimination between primary lung cancer and lung metastases due to another primary malignancy

The second question focused on the differentiation between primary lung cancer and metastases due to another primary malignancy. It is evident from Figure 1 that the two markers NSE and ProGRP differentiate best between these two groups. For NSE, the highest value in the group of lung metastases was 43 ng/mL, for ProGRP it was 55.4 pg/mL, this value is even lower than the highest value in the group of benign lung diseases (ProGRP: 244 pg/mL). CEA (highest value for metastases: 2861 ng/mL), though being statistically significant, is not able to discriminate between primary lung cancer and lung metastases due to another primary malignancy. For CYFRA 21-1 (highest value for metastases: 30.4 ng/mL), the situation is improved but remains non-significant.

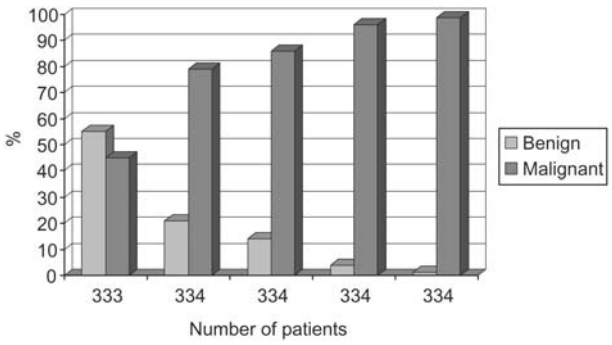

Figure 3 Leave-one-out validated score divided in quintiles: percentage of patients with benign and malignant lung diseases.

Support of histological classification NSCLC versus SCLC

For this purpose, the AUC and the 100% specificity were calculated and illustrated by ROC. The superiority of the two markers NSE and ProGRP was evident. For NSE, an AUC of 0.802 was calculated, for ProGRP the AUC was only slightly lower (0.759). For the calculation of the ROC curve for CYFRA 21-1, we used the reciprocal value, because a high value of CYFRA 21-1 is significant for NSCLC. The AUC of the two tumour markers CEA (0.494) and CYFRA 21-1 (0.568) was negligibly small. Regarding the 100% specificity of the single markers, only ProGRP and NSE reached 100% specificity. The best result was

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reached by ProGRP with 12.8%, whereas NSE reached 6.2%.

A further regression model was used to analyse the histological differentiation power into NSCLC or SCLC of the combination of the four tumour markers. In this model, the three tumour markers CYFRA 21-1, NSE and ProGRP provided significant differentiation between NSCLC and SCLC and thus were included in the final analysis (Table 6). The score was calculated as follows:

$$\text{Score} = -0.8117 \times \log(\text{CYFRA21-1}) + 1.5727 \times \log(\text{NSE}) + 0.6975 \times \log(\text{ProGRP}) - 7.3405.$$

Figure 4 illustrates the calculated ROC curve for the score compared with the ROC curves of the single markers. The AUC of the score reached with 0.836 the best result compared to the single used markers. Even the 100% specificity reached with 22.9% higher values than the single markers as reported in the univariate analysis.

Discussion

A number of biomarkers have been described to be of potential diagnostic relevance for lung cancer. The biomarkers most frequently investigated include CEA, CYFRA 21-1 and NSE, but also squamous cell carcinoma antigen (SCCA), cancer antigen 125 (CA 125) and tissue polypeptide antigen (TPA). More recently a new biomarker was included in many investigations, namely ProGRP, which has become of significant diagnostic relevance for SCLC.

Based on the various histological lung cancer subtypes, it is evident that in contrast to other solid tumours a panel of biomarkers is needed to reach a satisfying high sensitivity for NSCLC and SCLC patients. Therefore, many investigations deal with different combinations of biomarkers [20–31].

This present investigation is based on the biomarkers CEA, CYFRA 21-1, NSE and ProGRP following locally issued guidelines (Tumorzentrum München, http://tumourzentrum-muenchen.de). These analyses are routinely performed in patients before primary surgery of lung cancer or in patients with suspicious signs of lung cancer. Here, we evaluated the additional diagnostic or differential diagnostic capacity of lung cancer biomarkers at the time of primary diagnosis.

Concerning the first clinical question if biomarkers are able to give diagnostic aid in the differentiation between

Table 6 Logistic regression for the discrimination between SCLC and NSCLC (Ln = natural logarithm).

Variable	Coefficient	Standard error	p	Odds ratio	95% Confidence limits
Ln CYFRA 21-1	–0.8117	0.2041	<0.0001	0.444	0.298–0.663
Ln NSE	1.5727	0.2456	<0.0001	4.820	2.978–7.800
Ln ProGRP	0.6975	0.1217	<0.0001	2.009	1.582–2.550
Constant	–7.3405	0.6874	<0.0001		

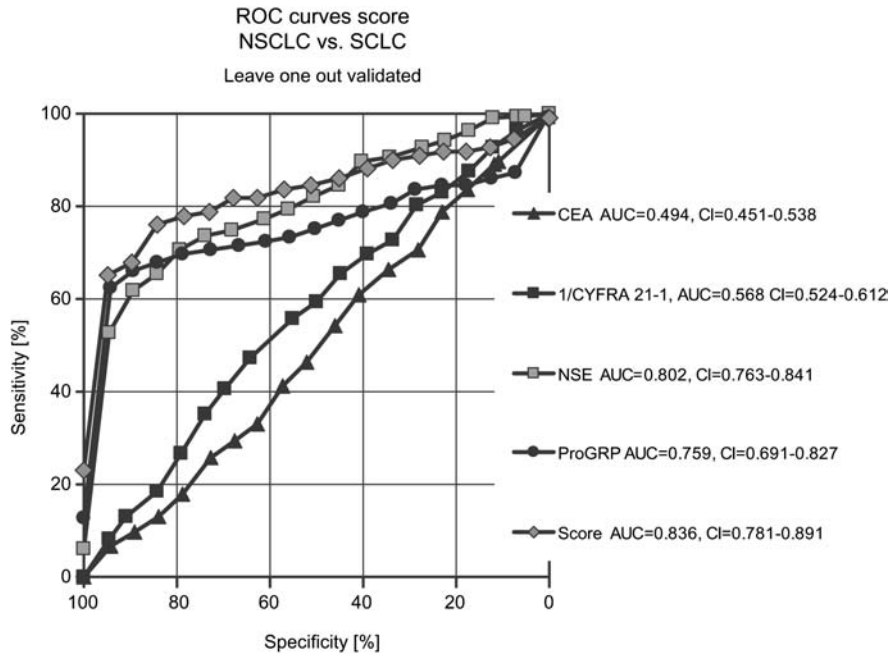

Figure 4 ROC curves and AUC of CEA, CYFRA 21-1, NSE and ProGRP and the established score for SCLC and NSCLC.

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benign and malignant lung tumours, there is no trial described in the literature which is based on a comparable high number of patients and focused from the outset on a histological subtype of lung cancer or a special stage of tumour disease. In addition, most investigations are only based upon univariate procedures.

Our findings revealed that very high concentrations of all four biomarkers are able to discriminate between benign and malignant lung tumours, corresponding to the clinical experience that from a certain level of these oncological biomarkers CEA, CYFRA 21-1, NSE and ProGRP the probability of a malignant solid tumour is very high. The most sensitive marker is CEA with 17% sensitivity, the combination of all four markers leads to a clear improvement with 50% sensitivity.

Plebani et al. [31] studied seven tumour markers (NSE, SCC, CEA, CYFRA 21-1, TPA (Tissue Polypeptide Antigen), TPM (Tissue Polypeptide Monoclonal Antigen) and TPS (Tissue Polypeptide Specific Antigen)) to evaluate their diagnostic efficiency in SCLC and NSCLC. Comparable with our results, this study showed that all tumour markers had significantly higher values in patients with lung cancer compared with those who suffer from benign lung diseases. ROC analysis revealed that the highest diagnostic accuracies in distinguishing benign from malignant lung diseases were achieved with TPM (81%, sensitivity 79% and specificity 84%), CYFRA 21-1 (72%, sensitivity 64% and specificity 86%), CEA (78%, sensitivity 89% and specificity 56%) and TPA (78%, sensitivity 82% and specificity 71%). Our results revealed comparable findings, although our aim was the very high specificity of $>$99% in order to avoid false positive results. As the former investigation was performed without fixed specificities, it is impossible to compare results in further detail.

We conclude from the data presented that the biomarkers CEA, CYFRA 21-1, NSE and ProGRP each reach 100% tumour specificity at very high levels and that their combined use is able to differentiate between benign and malignant lung diseases with a 50% sensitivity.

The next clinical question examined if it is possible to differentiate by using biomarkers between a primary lung tumour or lung metastases from another primary malignancy. As expected, CEA and CYFRA 21-1 are unable to reach the high specificity as they are released by almost all other solid tumours. However, the combination of CYFRA 21-1 and ProGRP allows, with a sensitivity of 21.2% and a specificity of 100%, to differentiate between primary lung cancer and lung metastases due to another primary malignancy. The combination of NSE and ProGRP reached a sensitivity of 17.6%, followed by the single use of ProGRP with 15.9%. The superiority of NSE and ProGRP to answer this question may be explained by the fact that NSE and especially ProGRP are mostly released by lung cancer, in contrast to CEA and CYFRA 21-1 being rather a marker for all malignancies. Therefore, we conclude that high NSE or ProGRP values are very suggestive of a primary lung cancer. This question has, to the best of our knowledge, not yet been addressed in the literature.

Having diagnosed a primary lung cancer it is imperative to provide histological differentiation between SCLC and NSCLC for its importance for prognostic and therapeutic reasons. Therefore, we studied the supportive power of tumour markers with regard to histology.

Concerning the differentiation of SCLC patients from NSCLC patients, high concentrations of CEA and CYFRA 21-1 could be observed predominantly within the group of NSCLC patients. Especially for CYFRA 2-1, this finding has been described previously by several investigators [21, 24, 32–35]. In our investigation, a CYFRA 21-1-value $>$100 ng/mL was only observed in patients suffering from NSCLC.

However, the best discrimination between NSCLC and SCLC could be achieved by using NSE and ProGRP, as previously described [18, 29].

In multivariate analysis, CYFRA 21-1, NSE and ProGRP turned out to be significant factors for the differentiation between SCLC and NSCLC patients. The corresponding score leads to improvement of the AUC from 0.802 for NSE as best single marker to 0.836 for the combination.

In conclusion, we state that the multivariate use of the biomarkers CEA, CYFRA 21-1, NSE and ProGRP leads to an increase of the diagnostic capacity for the discrimination between benign and malignant lung tumours, as well as for the discrimination between NSCLC and SCLC.

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