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Original Paper

Diagnostic and Prognostic Value of Cyfra 21-1 Compared with Other Tumour Markers in Patients with Non-small Cell Lung Cancer: A Prospective Study of 116 Patients

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The diagnostic value of Cyfra 21-1 in non-small lung cancer (NSCLC) has been established, but few studies have focused on its prognostic value. The aim of this study was to compare that of carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, CA 125, neuron-specific enolase and squamous cell carcinoma antigen. 116 patients with unresectable ($n = 88$) or resectable ($n = 28$) NSCLC were prospectively monitored from diagnosis, for a median of 14.4 months. All patients underwent tumour-marker determinations before treatment, then every 3 months. Their diagnostic value was studied using ROC (receiver operating characteristic) curves, based on control measure in 23 patients with benign lung diseases. The prognostic analysis was based on overall survival as the main endpoint. The diagnostic value of Cyfra 21-1 was confirmed, with a sensitivity of 54% and a specificity of 96% at a cut-off value of 3.3 ng/ml. At diagnosis, in the 88 non-surgical NSCLC, besides the presence of metastases ($P = 0.017$), Cyfra 21-1 ($P = 0.017$) and CA 125 ($P = 0.03$) were related to outcome. Elevated levels of Cyfra 21-1 at any time during the disease course was selected by multivariate analysis as additional predictors of poor survival.

Key words: non-small cell lung cancer, tumour markers, Cyfra 21-1, survival, prognosis

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INTRODUCTION

THE PROGNOSIS of non-small cell lung carcinoma (NSCLC) remains poor, with a 5-year survival rate after complete resection ranging from 60% to 85% in stage I, 45% in stage II and 30% in stage IIIa [1]. Only 5% of patients at clinical stage IIIb and almost none at clinical stage IV survive at 5 years [1]. In patients eligible for surgery, the main reported prognostic factors are disease stage [2–5] and histological subtype [5]. In non-surgical patients, outcome appears to be related to the Karnofsky performance index, disease stage and cell type [5–7].

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Biological prognostic factors have also been described. Two serum tumour markers, carbohydrate antigen (CA) 125 [8, 9] and squamous cell carcinoma antigen (SCC), have been reported to be of prognostic value [10, 11]. In a series of 96 squamous cell lung carcinomas, increases in carcinoembryonic antigen (CEA) and SCC levels were shown to be associated with a poor survival [10]. Niklinski and associates [11] reported a positive correlation between stage and SCC levels. Recently, Cyfra 21-1, a fragment of cytokeratin 19, was investigated in NSCLC, with reasonable diagnostic performances [12–22]. Using a threshold of 3.3 ng/ml, Wieskopf and associates [22] recently showed a 68% sensitivity and 94% specificity in squamous cell lung carcinoma, compared with 59% and 94%, respectively, in NSCLC, and only 19% and 94%, respectively, in small cell

lung carcinoma [22]. Pujol and associates [12] demonstrated 63% sensitivity and 91% specificity of Cyfra 21-1 in squamous cell lung carcinoma, using a threshold of 3.6 ng/ml; in NSCLC, these figures were 56% and 89%, respectively. Finally, the prognostic value of Cyfra 21-1 level, measured at diagnosis, on overall survival has been reported by several authors, either in NSCLC [22] or only in squamous cell lung carcinoma [12].

The aims of this study of NSCLC, regardless of the histological subtype, were: (1) to confirm the diagnostic sensitivity and specificity of Cyfra 21-1, (2) to identify a possible relationship between Cyfra 21-1 and other tumour markers, and (3) to assess the prognostic value of Cyfra 21-1 not only at diagnosis but also during follow-up, after completion of non-surgical treatment.

PATIENTS AND METHODS

Patient population

Between May 1992 and April 1995, 116 consecutive untreated patients (89 males, 27 females, age range: 39–84 years, median age: 60 years) with histologically confirmed NSCLC, referred to the Chest Department of Hôtel-Dieu University Hospital (Paris, France), were prospectively enrolled in this study. There were 49 squamous cell carcinomas, 51 adenocarcinomas, 15 large-cell carcinomas and one squamous and neuroendocrine carcinoma. Besides biomarker assays, all patients underwent a physical examination, fibre-optic bronchoscopy, chest radiography, computed tomographic scan of the chest, brain and liver, and isotope bone scan before treatment. NSCLC was staged according to the UICC TNM classification with post-surgical anatomical/pathological staging for resectable patients and clinical staging for unresectable patients [2]. There were 15 stage I, three stage II, 18 stage IIIa, 25 stage IIIb and 55 stage IV tumours (Table 1). Serum levels of six tumour markers, namely Cyfra 21-1, CEA, CA 19-9, CA 125, neuron-specific enolase (NSE) and SCC, were also determined.

The six tumour markers were also measured in 23 patients with benign lung diseases simulating cancer, comprising 13 cases of subacute pneumonia, five of solitary pulmonary nodule and five of interstitial pneumonia.

Treatment of each patient was decided on by a medical panel including a chest surgeon, radiologists, a radiotherapist and a medical oncologist, according to routine clinical and biological findings; the panel was blinded to serum tumour marker levels.

28 patients with resectable NSCLC (11 stage I, 3 stage II, 13 stage IIIa, 1 stage IIIb) underwent surgery, and resection was always complete. The remaining 88 patients who had non-resectable NSCLC (5 stage IIIa with bulky involvement, 24 stage IIIb and 55 stage IV) or who could not undergo surgery because of poor pulmonary function (4 stage I patients) were given chemotherapy (57 cases), palliative radiation therapy (14 cases), combined chemotherapy and radiation therapy (4 cases) or supportive care only (13 cases).

Biomarker assays

A blood sample was obtained from each patient at presentation and every 3 months after treatment completion; serum was stored at −20°C until use. All serum samples were assayed by technicians unaware of clinical data. Each sample was tested either in duplicate or both pure and diluted.

Cyfra 21-1 was assayed by using a kit from Cis-Bio International (Elsa; Cyfra 21-1) according to the one-step sandwich method. CEA, CA 125, CA 19-9, SCC and NSE

Table 1. Baseline characteristics of the 116 included patients at diagnosis, according to the resectability of the NSCLC

	Resectable and operable NSCLC ($n = 28$)	Unresectable or non-operable NSCLC ($n = 88$)
Male	25 (89%)	64 (73%)
Median age (range), years	57.5 (41–72)	60.5 (39–84)
Histology		
Squamous cell carcinoma	14 (50%)	35 (40%)
Adenocarcinoma	14 (50%)	37 (42%)
Large-cell carcinoma	0	15 (17%)
Squamous and neuro-endocrine carcinoma	0	1 (1%)
TX		2 (2%)
1	8 (28%)	4 (5%)
2	12 (43%)	17 (19%)
3	7 (25%)	15 (17%)
4	1 (4%)	50 (57%)
NX	0	1 (1%)
0	14 (50%)	28 (32%)
1	6 (21%)	1 (1%)
2	8 (29%)	34 (38%)
3	0	24 (27%)
M0	28 (100%)	33 (38%)
1	0	55 (62%)
Stage		
I	11 (39%)	4 (5%)
II	3 (11%)	0
IIIa	13 (46%)	5 (6%)
IIIb	1 (4%)	24 (27%)
IV	0	55 (63%)

were measured by using radioimmunoassay kits (CEA by Behring RIA gnost, CA 19-9 and NSE by Cis-Bio International, CA 125 by BYK Mallinckrodt, SCC by Abbott). The CEA, CA 125 and CA 19-9 kits were two-step sandwich assays, while the NSE and SCC kits were one-step sandwich assays.

The upper limits of normal laboratory values were 3.3 ng/ml for Cyfra 21-1, 5 ng/ml for CEA, 35 U/ml for CA 125, 45 U/ml for CA 19-9, 3 ng/ml for SCC and 12.5 ng/ml for NSE.

Endpoints

The main endpoint was overall survival. Disease-free survival was also analysed in the surgery group. In the unresectable group, the Cyfra 21-1 response was evaluated by comparing values between pretreatment and after restaging; a response was defined as a return to a value below the cut-off (see below) or a fall of at least 50% relative to the pretreatment value.

Statistical analysis

First, using the entire data set for the patients and controls, we compared the diagnostic value of the six biomarkers, i.e. their sensitivity (proportion of patients with marker values above the cut-off) and specificity (proportion of controls with marker values below the cut-off), using receiver operating characteristic (ROC) curves [23]. This was rerun for discriminating those with resectable disease and others among patients with NSCLC. The optimal Cyfra 21-1 cut-off was defined as the intersection point of the sensitivity and specificity curves. Correlations between biomarkers at diagnosis were identified by using Spearman's coefficient, while distributions between subgroups were compared by using the Wilcoxon test. No specific analysis was made according to histological type, owing to the small sample size.

A prognostic study was also conducted using overall survival as the endpoint, which was computed from the date of diagnosis and estimated by the product-limit method [24]. However, given the different outcomes between resectable and non-resectable NSCLC [5–7], we focused on the subset of unresectable NSCLC. The prognostic value of baseline characteristics of the patient (age, sex, smoking history), NSCLC (TNM, stage, histological type), or the six tumour biomarkers (categorised according to the optimal cut-off point as defined on ROC curves or to the normal range in our laboratory) was tested separately by the logrank test [25]. A Cox regression model [26] was used to summarise prognostic information, a backward step-down procedure selecting those variables adding to others prognostic information on the basis of the likelihood ratio test. Prognostic analyses were rerun using the biomarker values obtained during follow-up, based on a Cox model with time-dependent covariates, including the time required to reach a Cyfra 21-1 level above 2.5 ng/ml [26].

Levels of significance are represented by P values derived from two-sided tests. A P value of 0.05 or less was considered to indicate statistical significance. SAS (Statistical Analysis System Inc., Carey, North Carolina, U.S.A.) and BMDP (Biomedical Computers Programs, University of California, Los Angeles, California, U.S.A.) software packages were used.

RESULTS

Pretreatment biomarker values

The distribution of initial biomarker values in the entire sample is shown in Table 2. Patients with benign and malignant lung disease differed in terms of mean Cyfra 21-1

Table 2. Tumour biomarker distribution at study entry (diagnosis) according to the benign or malignant nature of the lung disease and resectability and operability of NSCLC

		NSCLC	
Disease	Benign lung disease (n = 23)	Resectable (n = 28)	Unresectable (n = 88)
Cyfra 21-1 (ng/ml) (mean ± S.D.)	1.15 ± 0.85	4.58 ± 6.4	15 ± 34
Median (range)	0.9 (0.4–3.6)	1.35 (0.5–27)	4.5 (0.2–240)
No. above normal cut-off (>3.3)	1 (4%)	10 (36%)	53 (60%)
CEA (ng/ml) (mean ± S.D.)	2.8 ± 1.2	27.1 ± 72.7	109 ± 404
Median (range)	2.55 (1.1–5.6)	4.05 (1.4–360)	7.4 (0.9–3310)
No. above normal cut-off (>5)	2 (9%)	11 (39%)	51 (58%)
CA 125 (U/ml) (mean ± S.D.)	42.7 ± 48.0	54.2 ± 121.4	455 ± 1977
Median (range)	27.5 (3–188)	18 (4–645)	98 (4–17980)
No. above normal cut-off (>35)	8 (35%)	9 (32%)	57 (65%)
CA 19-9 (U/ml) (mean ± S.D.)	19.0 ± 13.8	28.5 ± 48.8	1918 ± 17611
Median (range)	18 (2–53)	14 (2–256)	17 (2–165 240)
No. above normal cut-off (>45)	2 (9%)	3 (11%)	16 (18%)
SCC (ng/ml) (mean ± S.D.)	0.70 ± 0.35	24.0 ± 112.6	1.89 ± 2.71
Median (range)	0.6 (0.3–1.6)	0.85 (0.1–598)	0.85 (0.1–17)
No. above normal cut-off (>3)	0	4 (14%)	18 (20%)
NSE (ng/ml) (mean ± S.D.)	7.2 ± 3.0	7.3 ± 2.5	12.4 ± 19.7
Median (range)	5.9 (4.4–16)	7.1 (3.9–15)	7.8 (2.8–150)
No. above normal cut-off (>12.5)	2 (9%)	2 (7%)	16 (18%)

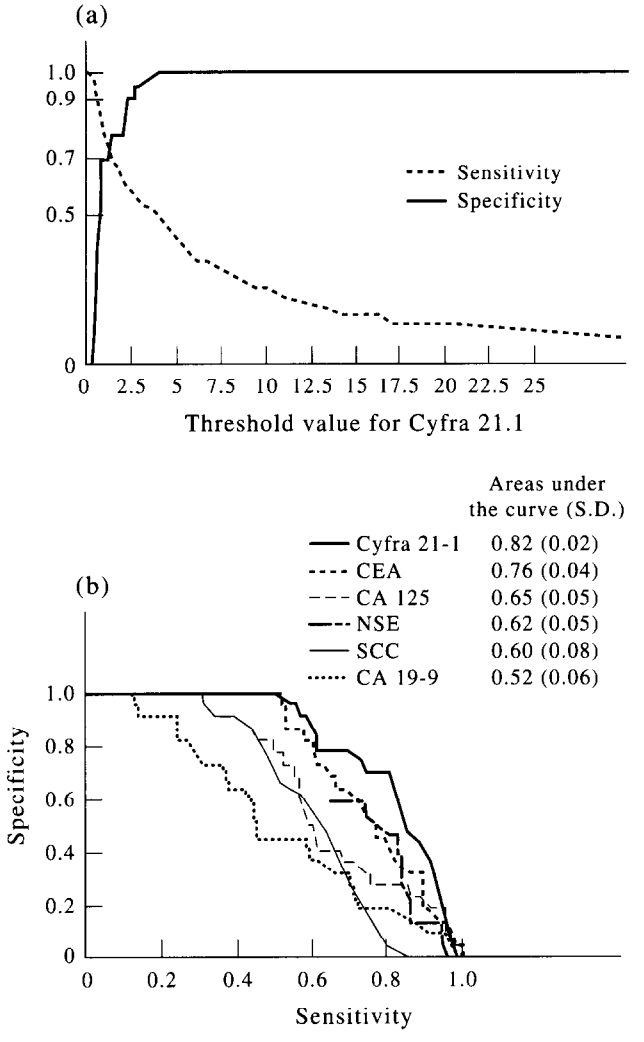

Figure 1. Value of Cyfra 21-1 compared with five other tumour markers in the diagnosis of NSCLC in 139 patients with lung disease. (a) The sensitivity and specificity in the diagnosis of NSCLC of Cyfra 21-1 versus the Cyfra 21-1 concentration. (b) Compares the areas under the ROC curves (with standard deviation, S.D.) obtained with Cyfra 21-1 to that of other markers at diagnosis.

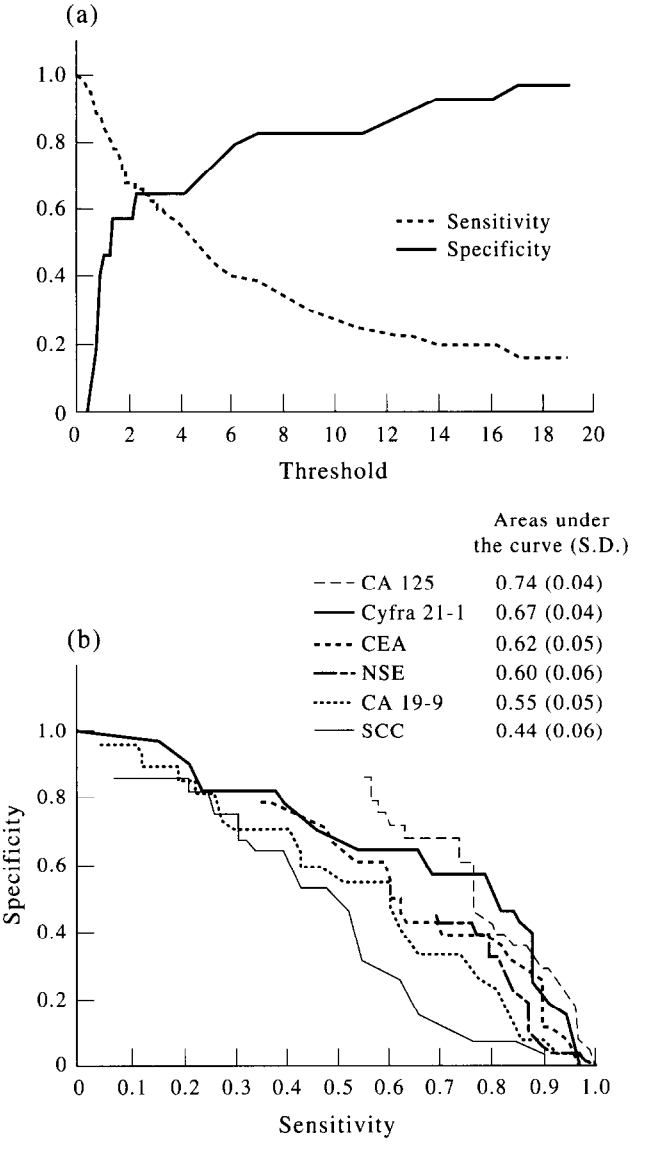

Figure 2. Value of Cyfra 21-1 compared with five other tumour markers in predicting the resectability and operability of NSCLC in 116 NSCLC patients. (a) The sensitivity and specificity of Cyfra 21-1 in the prediction of resectability and operability of NSCLC versus the Cyfra 21-1 concentration. (b) The areas under the ROC curves (with standard deviation, S.D.) obtained with Cyfra 21-1 with other markers at diagnosis.

levels ($P < 0.0001$) and CEA levels ($P = 0.0002$), while no significant difference in the other markers was observed. The distribution of Cyfra 21-1 also differed between benign lung disease and resectable NSCLC, as did that of CEA.

To study the diagnostic performance of the Cyfra 21-1 level, the sensitivity and specificity of Cyfra 21-1 were plotted against the Cyfra 21-1 concentration (Figure 1). Using a threshold of 3.3 ng/ml, sensitivity and specificity were 0.54 and 0.96, respectively; the intersection point was at a lower value of 1.25 ng/ml, at a sensitivity and specificity of approximately 0.70. At 95% specificity, the sensitivity was similar for Cyfra 21-1 and CEA (approximately 0.55) followed by SCC (0.32) and CA 19-9 (0.15), which clearly ruled out all markers but Cyfra 21-1 and CEA for diagnostic purposes. This was confirmed by computation of the areas under the ROC curve (AUC) for these markers, with the highest AUC for Cyfra 21-1 compared to that of the other markers (Figure 1).

Regarding the 116 cases of NSCLC at baseline, mean Cyfra 21-1 ($P = 0.007$), CA125 ($P = 0.0001$), NSE ($P = 0.06$) and CEA ($P = 0.07$) levels differed between the 28 resectable patients and the 88 non-surgical patients (Table 2). At a threshold value set at 2.5 ng/ml, defining the intersection point, Cyfra 21-1 had a sensitivity and specificity of 65% (Figure 2). An optimal cut-off value of 2.5 ng/ml was thus retained for Cyfra 21-1, while the optimal cut-off was obtained in the same way for the other markers (5 ng/ml for CEA, 35 U/ml for CA 125, 17 U/ml for CA 19-9, 7.6 ng/ml for NSE and 1 ng/ml for SCC). However, none of the markers clearly segregated resectable and non-surgical patients, and all the areas under the ROC curve remained below 0.75 (Figure 2).

At diagnosis, Cyfra 21-1 was weakly but significantly linked to CA 19-9 (Spearman's coefficient, $r = 0.03$, $P = 0.04$) and SCC ($r = 0.22$, $P = 0.22$), but not to CEA

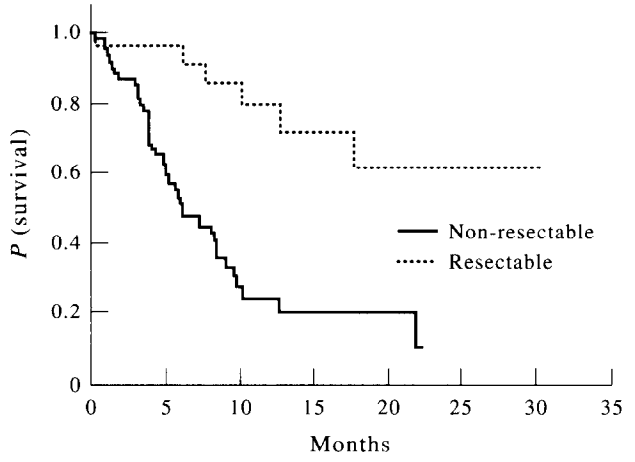

Figure 3. Overall survival from diagnosis according to the initial resectability of the NSCLC.

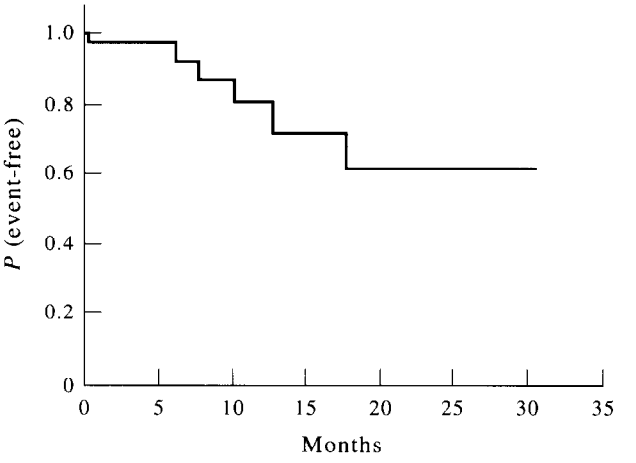

Figure 4. Disease-free survival from diagnosis in resectable NSCLC.

($r = 0.18$, $P = 0.10$), CA 125 ($r = 0.005$, $P = 0.96$), or NSE ($r = 0.15$, $P = 0.19$).

Endpoints

At the reference date of 15 May 1995 the median follow-up was 14.4 months; 46 patients had died, 40 with unresectable NSCLC and six after resection (Figure 3). The median survival time was not reached in the resectable group, but was estimated to be 6 months in the non-resectable group ($P = 0.0001$).

Surgery group. Disease-free survival among the 28 patients with resectable NSCLC is shown in Figure 4. 9 patients had relapses, which were locoregional (4 patients), metastatic (4 patients) or both (1 patient), after a median of 19 months.

Non-resectable group. The univariate prognostic analysis of baseline characteristics selected four factors at the 5% level (Table 3). Patients with metastasis at diagnosis had a 5 months median survival time compared to 9.7 months in those without ($P = 0.001$); median survival time was 5 months in stage IV patients compared to 22 months in stage IIIb, while it was 6 months in the other patients (4 of the 5 stage IIIa unresectable patients but none of the 4 stage I–II unresectable patients died). Besides the prognostic information provided by metastases and stage, two markers, Cyfra 21-1 ($P = 0.02$) and CA 125 ($P = 0.007$), were significantly associated with outcome. NSE ($P = 0.09$) and CEA ($P = 0.13$) were close to statistical significance, while SCC ($P = 0.63$) and CA 19.9 ($P = 0.46$) were not. However, to compare the prognostic value of the six tumour markers, they were introduced into the Cox model jointly with metastasis (Table 4). Of the previous seven variables, metastasis ($P = 0.017$), Cyfra 21-1 level ($P = 0.017$) and CA 125 ($P = 0.03$) summarised the prognostic information (Table 4). When adjusted for metastases and baseline CA 125 values, patients with Cyfra 21-1 levels $\geqslant 2.5$ ng/ml had a 2.5-fold higher risk of death compared with patients with lower levels (< 2.5 ng/ml).

During follow-up, four biomarker levels were associated with outcome when tested separately: Cyfra 21-1 ($P = 0.0001$), CA 125 ($P = 0.0006$), CEA ($P = 0.006$) and NSE ($P = 0.018$). These results were slightly modified by adjustment for metastases at baseline. In 34 (59%) of the 58 patients with baseline Cyfra 21-1 levels above 2.5 ng/ml, levels decreased after treatment to 2.5 ng/ml or less. At least once during follow-up, the Cyfra 21-1 level reached 2.5 ng/ml in 26 patients, 20 (77%) of whom died, compared with 20 (32%) deaths among the 62 patients whose Cyfra 21-1 level remained below 2.5 ng/ml after treatment. When incorporated simultaneously into a Cox model, together with the baseline prognostic information yielded by metastases, two variables were retained by the stepwise procedure as significantly adding to others' prognostic information, namely metastasis at diagnosis ($P = 0.02$, likelihood ratio test) and follow-up values of Cyfra 21-1 ($P = 0.0001$); CA 125 ($P = 0.105$) and NSE ($P = 0.098$) were non-significant. Patients whose Cyfra 21-1 level increased to 2.5 ng/ml or more had a 6-fold higher risk of death as compared with those whose levels were <2.5 ng/ml after adjustment for the other covariates (Table 4).

DISCUSSION

This study confirms that Cyfra 21-1 has good specificity in NSCLC. At a cut-off value of 3.3 ng/ml, the specificity was 96% while the sensitivity was only 54%, in accordance with previous reports [13, 15, 19, 22]. Almost half the patients with NSCLC had Cyfra 21-1 values within the normal range for our laboratory. Clearly, Cyfra 21-1, like other well-known markers, has no place in NSCLC screening programmes. In our series, the best compromise Cyfra 21-1 value between sensitivity and specificity was 1.25 ng/ml, with a true-positive rate (sensitivity) of 70% and a false-positive rate of 30% (i.e. 100 – specificity). Evaluation of the panel of tumour markers from the ROC curves clearly showed that Cyfra 21-1 was superior to CEA, NSE, CA 125, SCC and CA 19-9 for the diagnosis of NSCLC, as previously reported [13, 14, 18, 21, 22]. Finally, there was a weak but highly significant correlation between Cyfra 21-1 and CA 19-9 and SCC, while no correlation was observed between CEA, CA 125 or NSE.

Serum levels of Cyfra 21-1, CA 125, NSE and CEA differ significantly according to NSCLC disease stage [13, 18] and resectability [12]. Nevertheless, none of these markers predicted resectability in our series. The optimal Cyfra 21-1 cut-off, which was retained for subsequent prognostic analysis, was 2.5 ng/ml. The 65% specificity and sensitivity of

Table 3. Unresectable or non-operable NSCLC: univariate prognostic analysis based on baseline information at NSCLC diagnosis

Covariate	Number of patients ($n = 88$)	Number of deaths ($n = 40$)	Median survival (months)	P value (logrank)
Gender				
Male	64	30	5.8	0.64
Female	24	10	6.0	
Age (years)				
<60	39	17	5.8	0.94
⩾60	49	23	7.3	
Smoking history				
Smoker	51	22	8.1	
Previous smoker	26	14	5.7	0.78
Non-smoker	11	4	6.0	
Histology				
Squamous cell carcinoma	35	16	8.3	
Adenocarcinoma	37	19	5.8	0.54
Other	16	5	5.7	
T (tumour size)*				
0-1-2	21	7	8.4	0.49
3-4	65	31	5.8	
N (lymph nodes)†				
0	28	13	5.0	0.12
1-2-3	59	26	8.3	
M (metastasis)				
0	33	9	9.7	0.001
1	55	31	5.0	
Stage				
I–IIIa	9	4	6.0	
IIIb	24	5	22.0	0.003
IV	55	31	5.0	
Cyfra 21-1 (ng/ml)				
<2.5	30	8	9.7	0.02
⩾2.5	58	32	5	
CEA (ng/ml)				
<5	37	13	8.4	0.13
⩾5	51	27	5.0	
CA 125 (U/ml)				
<35	31	10	10.1	0.007
⩾35	57	30	5.1	
CA 19-9 (U/ml)				
<17	47	23	5.7	0.46
⩾17	41	17	7.3	
SCC (ng/ml)				
<1	50	21	6.0	0.63
⩾1	38	19	5.8	
NSE (ng/ml)‡				
<7.6	40	17	9.6	0.09
⩾7.6	43	20	5.0	

* 2 patients had TX. † 1 patient had NX. ‡ Data not available for 5 patients.

Cyfra 21-1 in this respect was not far from the 69% accuracy of tissue polypeptide antigen (TPA) and was similar to that of the computed tomograph in the report by Buccheri and associates [27].

The prognostic value of Cyfra 21-1 at diagnosis was assessed in the subset of patients with advanced and non-resectable NSCLC. Univariate analysis of survival in this group clearly demonstrated the prognostic value of Cyfra 21-1, whatever the cut-off value (3.3 ng/ml or 2.5 ng/ml), as well as that of CA 125. Moreover, multivariate analysis retained Cyfra 21-1 and CA 125 as additional prognostic factors to stage. These findings are consistent with published studies on the prognostic value of Cyfra 21-1 in squamous cell carcinoma [12] and NSCLC of all histological types [22]. The prognostic value of CA 125 was demonstrated by Diez and associates [9] in resectable NSCLC but, to our knowledge, this has never been proven in advanced NSCLC. We found that these two markers were

Table 4. Unresectable and non-operable NSCLC: multivariate prognostic analysis incorporating both baseline and follow-up information

Model	Estimated relative risk of death	*P*-value (log likelihood ratio test)
Baseline information		
Metastasis	2.58	0.017
Cyfra 21-1 ⩾ 2.5 ng/ml	2.46	0.017
CEA ⩾ 5 ng/ml		0.55
CA 125 ⩾ 35 U/ml	2.30	0.034
CA 19.9 ⩾ 17 U/ml		0.12
SCC ⩾ 1 ng/ml		0.75
NSE ⩾ 7.5 ng/ml		0.84
Baseline and follow-up information		
Baseline metastasis	2.6	0.02
Cyfra 21-1 ⩾ 2.5 ng/ml	5.9	0.0001
CEA ⩾ 5 g/ml		0.40
CA 125 ⩾ 35 U/ml	1.9	0.105
NSE ⩾ 7.5 ng/ml	1.8	0.098

both associated with outcome in advanced non-surgical NSCLC. Finally, serial measurement of Cyfra 21-1 during the course of the disease provided prognostic information in our series, in addition to that given by baseline metastatic status, NSE and CA 125. In practice, both at diagnosis and during the course of the disease, NSCLC patients with serum levels of Cyfra 21-1 above 2.5 ng/ml would potentially be at an increased risk of death.

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