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CYFRA21-1 can predict the sensitivity to chemoradiotherapy of non-small-cell lung carcinoma

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Abstract

Background: The increasing panel of systemic therapies enables the individual management of lung cancer patients, even in advanced stages. However, predictive tools indicating the efficacy of chemoradiotherapy (CRT) are badly needed.

Aims: To determine the tumour markers for predicting the therapeutic effect in non-small-cell lung carcinoma (NSCLC) patients treated with CRT.

Methods: The serum levels of cytokeratin 19 fragment antigen 21-1 (CYFRA21-1), neurone-specific enolase (NSE) and carcinoembryonic antigen (CEA) were measured before CRT by enzyme-linked immunosorbent assays, while the tumour responses were assessed according to the World Health Organization (WHO) response criteria. The relationships between pretreatment expression of CYFRA21-1, NSE, CEA and the effectiveness of CRT were analysed.

Results: The complete response (CR) rate of the primary tumours estimated by computed tomography in patients with high levels of CYFRA21-1 was 2.9% (2/68) while in cases with low CYFRA21-1 it was 20.3% (12/59) ($p = 0.005$). The effective rates (CR+PR) in CYFRA21-1 high and low groups were 52.9% (36/68) and 72.9% (43/59), respectively ($p = 0.022$).

Conclusions: CYFRA21-1 may be a reliable surrogate marker of CRT efficacy in patients with NSCLC.

Keywords: *Non-small-cell lung carcinoma; chemoradiotherapy; cytokeratin 19 fragment antigen 21-1; carcinoembryonic antigen; neurone-specific enolase*

Introduction

Lung cancer is one of the most life-threatening cancers worldwide (Jemal et al. 2006). Its devastating incidence and clinical seriousness have stimulated innumerable studies directed toward any possible approach (Ferrigno et al. 1995). Research on predictive factors of sensitivity of tumours to chemoradiotherapy (CRT) (short-term therapeutic effect) in lung carcinoma is of great importance because it potentially leads to a better and perhaps tailored management of patients. Some serum tumour markers (TMs) may be helpful in the early assessment of the extent of the disease, and in monitoring the tumour growth (or tumour volume reduction) once cancer has been diagnosed and treatment started. They may also give insight into histogenesis, inter-relationships and biological behaviour of tumours (Ferrigno et al. 1994). Compared with imaging techniques, the TMs can not only mirror the cancer activity and metabolism, but also take into account the heterogeneity of the tumour tissue containing active, silent, apoptotic and necrotic parts (Holdenrieder et al. 2004, 2006, Vollmer et al. 2003, Werner-Wasik et al. 2001). Meanwhile, the former can only monitor the macroscopic changes of tumour mass. Accordingly, changes of tumour size are often detected only after several cycles of therapy (Holdenrieder et al. 2004, Werner-Wasik et al. 2001, Vansteenkiste et al. 2004). However, many TMs can be evaluated as predictive factors, either alone or in combination with other histopathological, biochemical and clinical variables (Buccheri & Ferrigno 1994). In non-small-cell lung cancer (NSCLC), carcinoembryonic antigen (CEA) and

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cytokeratin 19 fragment antigen 21-1 (CYFRA21-1) proved to be sensitive and valuable biomarkers for estimation of diagnosis, prognosis and therapy monitoring (Holdenrieder et al. 2004, 2006, Molina et al. 2003, Pujol et al. 2004). CEA and CYFRA 21-1 responses appeared to be reliable surrogate markers of chemotherapy efficacy in patients with advanced NSCLC (Ardizzoni et al. 2006). Icard et al. (1994) demonstrated that patients who undergo resection of stage I or II NSCLC with preoperative CEA levels under 30 ng ml^{-1} have a significantly longer survival than patients with CEA above 30 ng ml^{-1}. Pujol et al. (1993) demonstrated in a prospective study of 165 patients that CYFRA21-1 has an independent prognostic value and its serum level helps predict the resectability of NSCLC. In multivariate analyses, increased serum levels of CYFRA21-1 were found to be significantly associated with a higher risk of recurrence (Niklinski et al. 1995). These observations are consistent with preliminary data from other authors (Reinmuth et al. 2002), who also found that CYFRA21-1 was an independent prognostic factor for survival in NSCLC patients with complete resection. However, there are no studies about TMs associated with sensitivity to CRT. Based on these promising results, we analysed in the present study patients with primary NSCLC during first-line CRT using biomarkers to estimate the sensitivity of tumours to CRT in order to validate their predictive roles.

Patients and methods

Patients

One hundred and twenty-seven patients who received CRT, referred to the Shandong Tumour Hospital between July 2002 and July 2009, were entered in the study. Eligibility criteria consisted of histologically proven and previously untreated NSCLC. In addition, the following standards were also included: (1) Karnofsky Performance Status (KPS) scale 70–100; (2) patients with adequate organ, bone marrow, liver and renal functions; (3) patients with no severe complications; (4) patients with a computed tomography (CT) or positron-emission tomography (PET)/CT scan of evaluable primary lesions pretreatment and post-treatment; (5) clinically diagnosed T1–4, N (any) and M (any) on the International Union Against Cancer (UICC) tumour–node–metastasis (TNM) classification; and (6) informed consents were obtained before treatment. All patients were given the same regimen of CRT.

Treatment schedule

All patients received two cycles of chemotherapy with vinorelbine/gemcitabine and cisplatin combined with radiotherapy. Radiotherapy was administered using conformal radiotherapy or intensity-modulated radiotherapy with 15 MV X-rays in 30 fractions with a total dose of 60 Gy.

Evaluation of response concerning the primary site

Tumour responses were assessed as the following: complete response (CR) for the primary tumour was defined as the complete disappearance of all measurable and assessable disease for more than 4 weeks; partial response (PR) was defined as a subjective decrease with >50% tumour regression for more than 1 month; no change (NC) as <50% reduction of the tumour; and progression of disease (PD) as a ≥25% enlargement of the tumour or the appearance of a new tumour. The evaluation of the response to treatment consisted of thoracic CT or PET/CT. Two kinds of categorizing methods were employed in our evaluation: (1) patients who were evaluated as PR, NC and PD were considered to be non-CR, while patients with CR were classified into the CR group; (2) patients who were evaluated as CR and PR were regarded as effective while NC and PD were defined as ineffective. The evaluation was performed 2 months after the treatment.

Biochemical measurements, serum sampling, enzyme immunoassay for CYFRA21-1, NSE and CEA assay

Blood samples were obtained by venipuncture before CRT. Each sample was centrifuged at 3000*g* for 5 min and then frozen at -80°C until use. Repeated thawing and freezing of samples was avoided. CYFRA21-1, NSE and CEA levels were measured by enzyme immunoassay kits (Roche Diagnostics, Basel, Switzerland) accordingly. The cut-off values of CYFRA21-1, NSE and CEA were defined as 3.4, 17 and 3.3 ng ml^{-1}, respectively, according to 95% confidence interval (CI) of non-cancer Chinese. Levels above the cut-off values were defined as high while below the value as low.

Statistical analysis

The χ^2 test and logistic regression analysis were used to evaluate the association between the responsiveness of primary lesions and clinical variables. Significance was defined as $p < 0.05$. Statistical analyses were conducted with SPSS 16.0.

Results

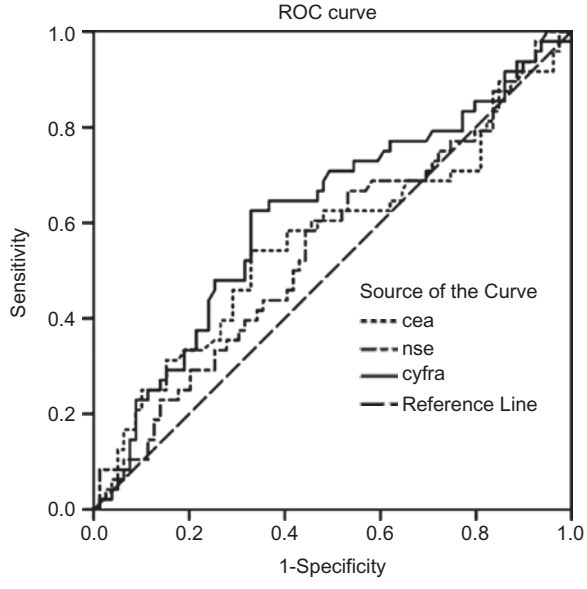
A group of 127 histologically proven NSCLC patients was evaluated and the characteristics of the patients are shown in Table 1. Fourteen CRs, 65 PRs, 40 NCs and eight PDs were found. There were 92 males and 35 females

with a median age of 59 years (range 30–80). Forty-one patients were the peripheral type while 86 patients were the central type. The value distribution of the serum levels of CEA, NSE and CYFRA21-1 within the various groups are shown in Figure 2A–C. Table 2 shows that the effectiveness of CRT was significantly associated with the serum levels of CYFRA21-1 before treatment; the difference of the CR rates between CYFRA21-1 high and low groups was significant ($p = 0.003$); the difference of the CR+PR rates between CYFRA21-1 high and low groups was also significant ($p = 0.028$). However, the effectiveness of CRT showed no significant association with the serum levels of CEA and NSE before treatment. The differences of the CR rates between the different location of the primary tumour were significant ($p = 0.036$); however, there were no differences of the CR+PR rates between the different location of the primary tumour ($p = 0.846$). Between KPS high and low groups, the difference of the CR+PR rates was significant ($p = 0.043$) while the difference of the CR rates was not ($p = 0.781$). Meanwhile, the differences of CR/CR+PR rates between the different tumour size were significant ($p = 0.015$, 0.036, respectively). The stage was also significantly correlated with the CR/CR+PR rate ($p = 0.016$, <0.001, respectively). Table 3 shows that the CR rate of CRT was significantly associated with the pretreatment levels of CYFRA21-1 ($p = 0.005$; 95% CI 2.093–60.567) by logistic regression analysis. The former was also significantly associated with the tumour size ($p = 0.046$). As shown in Table 4, the CR+PR rates were

Table 1. Characteristics of 127 patients with non-small-cell lung cancer.

	Patients	
Characteristic	*n*	Constituent ratio (%)
Sex		
Male	92	72.4
Female	35	27.6
Age		
≤60 years	69	54.3
>60 years	58	45.7
KPS		
≤80	69	53.5
>80	58	46.5
Location		
Peripheral	41	31.5
Central	86	68.5
Histological types		
SCC	58	45.7
AdenoCA	69	54.3
T stage		
T 1	23	18.1
T 2	37	29.1
T 3	38	29.9
T 4	29	22.8
N stage		
N 0	21	16.5
N 1	13	10.2
N 2	61	48.0
N 3	32	25.2
M stage		
M 0	85	66.9
M 1	42	33.1
CYFRA21-1 (ng ml^{-1})		
≤3.3	59	46.5
>3.3	68	53.5
CEA (ng ml^{-1})		
≤3.4	58	45.7
>3.4	69	54.3
NSE (ng ml^{-1})		
≤17	33	26.0
>17	94	74.0
Tumour size (cm)		
≤3	42	33.0
3–5	50	39.4
>5	35	27.6
UICC stages		
I	5	3.9
II	14	11.0
III	66	52.0
IV	42	33.1

KPS, Karnofsky Performance Status; SCC, squamous cell carcinoma; adenoCA, adenocarcinoma; CEA, carcinoembryonic antigen; NSE, neurone-specific enolase.



Area Under the Curve

Variable	Area	*P* value	95% Confidence Interval	
cea	.563	.238	.455	.671
nse	.550	.345	.446	.654
Cyfra21-1	.616	.028	.513	.719

Figure 1. Receiver operating characteristic curves for serum levels of CYFRA21-1, carcinoembryonic antigen (CEA) and neurone-specific enolase (NSE) in the group of patients with complete response + partial response (CR+PR) in relation to the effectiveness of chemoradiotherapy (CRT).

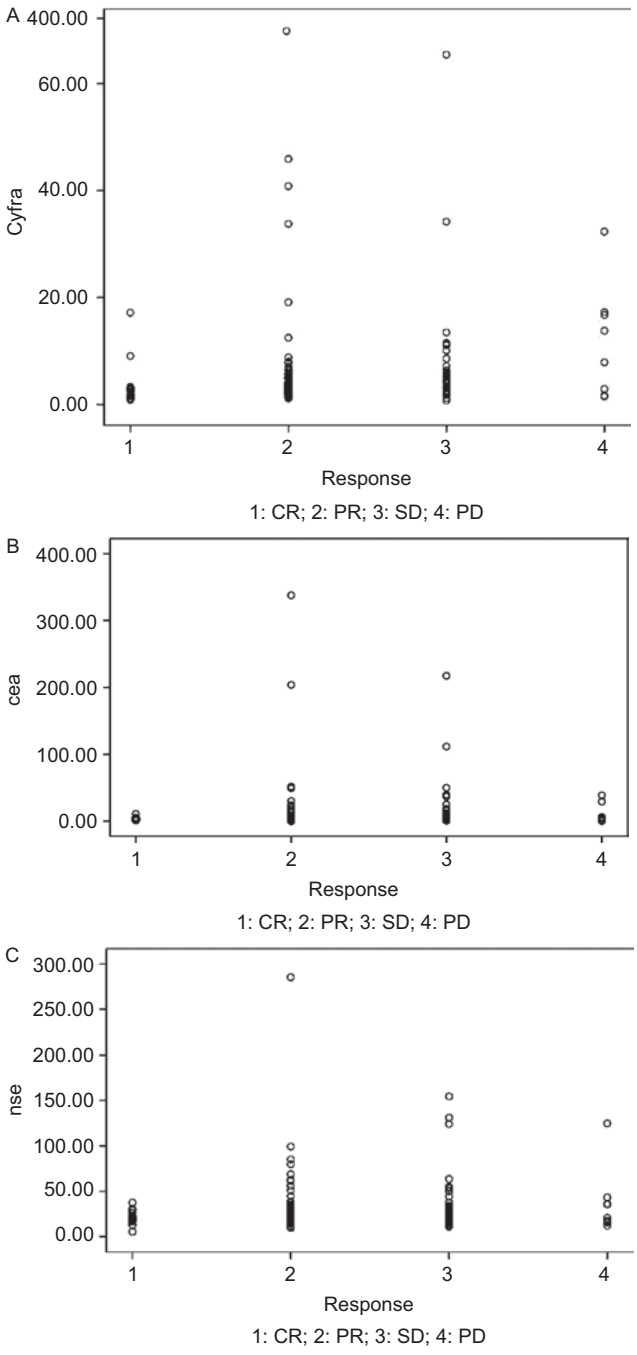


Figure 2. Dot plots illustrating the value distribution of the serum level of (A) carcinoembryonic antigen (CEA), (B) neurone-specific enolase (NSE) and (C) CYFRA21-1 within the various groups.

also significantly associated with the pretreatment levels of CYFRA21-1 ($p = 0.022$, 95% CI 1.157–6.534). Meanwhile, the former was significantly associated with the tumour size ($p = 0.037$), M stage ($p = 0.019$), KPS ($p = 0.031$) and UICC stage ($p = 0.028$). That is to say patients with high CYFRA21-1 were less sensitive to CRT. Table 5 shows the relationships between serum levels of CYFRA21-1, NSE, CEA and TNM stage in 127 patients with NSCLC. There was no significant association between the serum levels of CYFRA21-1, NSE, CEA and TNM stage. Figure 1 shows the receiver operating characteristic (ROC) curves for serum levels of CYFRA21-1, CEA and NSE in the group of patients with CR+PR in relation to the effectiveness of CRT. The sensitivity, specificity and positive and negative predictive values of the TMs are shown in Table 6. CYFRA21-1 showed a significant but minor predictive value for the sensitivity of tumours to CRT ($p = 0.028$, area under the curve (AUC) 0.616).

Discussion

TMs can be helpful in screening, early diagnosis of cancer, and in the initial assessment of the extent of disease (Ferrigno et al. 2003). For a long time, people have focused on the applications of diagnosis and prognosis of CYFRA21-1. Numerous investigators have reported that CYFRA21-1 could aid in diagnosis. It is the most sensitive biomarker in NSCLC, particularly for squamous cell carcinoma (SCC) (Cho et al. 2007, Schneider et al. 2003, Lamy et al. 2000). The prognostic significance of CYFRA21-1 for NSCLC has also been reported; both univariate and multivariate analyses demonstrated that CYFRA21-1 was significantly correlated with prognosis of patients with p-stage I NCSLC (Hatzakis et al. 2002). Meanwhile, a meta-analysis of pooled data from nine centres demonstrated CYFRA21-1 to be an independent prognostic factor in both early and advanced NSCLC, which confirmed earlier studies demonstrating its prognostic relevance (Kulpa et al. 2002, Hatzakis et al. 2002). The diagnosis and prognosis roles of CYFRA21-1 have been confirmed; however, little is known about its usefulness for the early prediction of therapy response. Previous studies only showed that in patients with advanced stage NSCLC undergoing chemotherapy, trends in CYFRA21-1 during the initial treatment phase predict the response to subsequent therapy (Cho et al. 2007). Our study retrospectively analysed biological and clinical variables in 127 NSCLC patients referred to our institution. We found that the CR/CR+PR rates in CYFRA21-1 high and low groups were significantly different. Meanwhile, the effectiveness rates of CRT were significantly associated with the pretreatment levels of CYFRA21-1 by logistic regression analysis. These results demonstrate the importance of CYFRA21-1 in predicting the sensitivity of tumours to CRT.

Diez et al. (1993) found that elevated NSE serum concentrations in patients with NSCLC conform to a high-risk group, with lower survival and greater probability of relapse after curative surgery. However, Ray et al. reported that serum NSE levels prior to treatment could not contribute to the prediction of an objective response of chemotherapy in NSCLC (Ray et al. 1998). In this study,

Table 2. Relationships between effectiveness of chemoradiotherapy and clinicopathological factors as well as serum levels of tumour markers.

	Effectiveness				Effectiveness			
Characteristics/markers	CR	Non-CR	χ^2	*p*-Value	CR+PR	NC+PD	χ^2	*p*-Value
Sex								
Male	11	81	0.296	0.756	58	34	0.1.00	0.838
Female	3	32			21	14		
Age								
≤60	7	62	0.119	0.781	46	23	1.279	0.276
>60	7	51			33	25		
KPS								
≤80	7	62	0.119	0.781	37	32	4.733	0.043
>80	7	51			42	16		
Location								
Peripheral	1	40	4.549	0.036	26	15	0.038	0.846
Central	13	73			53	33		
Histological types								
SCC	9	49	2.198	0.163	41	17	3.269	0.098
AdenoCA	5	64			38	31		
T stage								
T 1	5	18	3.630	0.272	15	8	0.135	0.987
T 2	2	35			23	14		
T 3	4	34			23	15		
T 4	3	26			18	11		
N stage								
N 0	3	18	4.905	0.179	12	9	0.692	0.875
N 1	0	13			9	4		
N 2	9	52			39	22		
N 3	2	30			19	13		
M stage								
M 0	10	75	0.147	0.701	56	29	1.479	0.247
M 1	4	38			23	19		
CYFRA21-1 (ng ml^{-1})								
≤3.3	12	47	9.749	0.003	43	16	5.343	0.028
>3.3	2	66			36	32		
CEA (ng ml^{-1})								
≤3.4	9	49	2.198	0.163	40	18	2.075	0.198
>3.4	5	64			39	30		
NSE (ng ml^{-1})								
≤17	4	29	0.054	0.817	21	12	0.039	0.844
>17	10	84			58	36		
Tumour size (cm)								
≤3	7	35	8.419	0.015	32	10	6.668	0.036
3–5	1	49			25	25		
>5	6	29			22	13		
UICC stages								
I	3	2	10.351	0.016	5	0	39.826	0.000
II	0	14			0	14		
III	7	59			51	15		
IV	4	38			23	19		

KPS, Karnofsky Performance Status; SCC, squamous cell carcinoma; adenoCA, adenocarcinoma; CEA, carcinoembryonic antigen; NSE, neurone-specific enolase.

although a slight tendency was observed, a statistically significant difference was not found. This may partly be because the sample was relatively small.

Studies have shown that CEA concentrations are particularly high in adenocarcinoma (adenoCA) and large-cell lung carcinoma (LCLC): if CEA is >10 mg l^{-1}, the presence of adenoCA or LCLC is very likely. CEA may also be helpful in the differential diagnosis of NSCLC, preferably in combination with CYFRA21-1 (Kulpa et al. 2000, Molina et al. 2003, 2005). However, the prognostic

Table 3. Logistic regression analysis of the relationships between effectiveness of chemoradiotherapy and clinicopathological factors as well as serum tumour markers in the group of patients with complete response.

Variables	*p*-Value	Hazards ratio	95% Confidence interval Lower	Upper
Sex	0.516	0.421	0.090	3.345
Age	0.627	0.236	0.172	2.887
KPS	0.905	0.014	0.266	4.468
Location	0.089	2.884	0.012	1.376
Histological types	0.175	1.843	0.612	14.884
T	0.119	2.435	0.851	4.160
N	0.334	0.933	0.233	1.641
M	0.313	1.018	0.022	3.413
Tumour size (cm)	**0.046**	3.988	0.447	0.992
CYFRA21-1 (ng ml^{-1})	**0.005**	7.954	2.093	60.567
CEA (ng ml^{-1})	0.426	0.633	0.129	2.374
NSE (ng ml^{-1})	0.339	0.914	0.423	12.154
UICC stages	0.329	0.953	0.491	8.363
Constant	0.916	0.011		

KPS, Karnofsky Performance Status; CEA, carcinoembryonic antigen; NSE, neurone-specific enolase.

Table 4. Logistic regression analysis of the relationships between effectiveness of chemoradiotherapy and serum levels of tumour markers in the group of patients with complete response + partial response (CR+PR).

Variables	*p*-Value	Hazards ratio	95% Confidence interval Lower	Upper
Sex	0.972	0.001	0.370	2.611
Age	0.141	2.164	0.801	4.757
KPS	0.031	4.641	0.161	0.917
Location	0.851	0.035	0.427	2.807
Histological types	0.057	3.62	0.971	7.182
T	0.649	0.207	0.567	1.424
N	0.338	0.917	0.786	2.016
M	**0.019**	5.471	1.365	34.051
Tumour size (cm)	**0.037**	4.36	1.016	1.663
CYFRA21-1 (ng ml^{-1})	0.022	5.249	1.157	6.534
CEA (ng ml^{-1})	0.479	0.501	0.298	1.765
NSE (ng ml^{-1})	0.891	0.019	0.34	2.558
UICC stages	**0.028**	4.824	0.121	0.887
Constant	0.56	0.339		

KPS, Karnofsky Performance Status; CEA, carcinoembryonic antigen; NSE, neurone-specific enolase.

impact of the preoperative CEA level has been controversial. Some authors have reported that CEA has prognostic value for patients after resection of NCSLC (Ando et al. 2001, Okada et al. 2004, Hotta et al. 2000, Icard et al. 1994). In contrast, other studies have found that an elevated preoperative CEA level is only marginally predictive or completely lacking in prognostic value (Reinmuth et al. 2002, Nisman et al. 1998, Foa et al. 1999). Kulpa et al. (2000, 2002) also demonstrated that the preoperative CEA level was unrelated to survival in patients with SCC. In addition, there are findings indicating that CEA expression by a tumour and elevated CEA level in the serum may predict refractoriness of the tumour to chemotherapy (Segawa et al. 1993). In our study, a statistically significant difference was not found between CEA high and low groups, and the CR/CR+PR rates of CRT were not significantly associated with the pretreatment levels of CEA. This may also be ascribed to the relatively small sample.

Studies have shown that smaller initial tumour size, as measured by largest tumour dimension, or tumour volume, is associated with better local control and survival than larger size (Maria et al. 2001). Maria et al. also reported that patients with smaller (≤45 cm^3) tumours achieved a longer median survival time (MST) and better progression-free survival (PFS) than did patients with larger (>45 cm^3) tumours. Age, sex, performance status, histological type, N stage, previous chemotherapy and maximal radiation dose were not significant (Maria et al. 2008). Trani et al. (2010) also reported that neither univariate nor multivariate analysis suggested that there was a significant difference in the response rates for adenoCA versus non-adenoCA or between squamous and non-squamous pathology. In the present study, the effectiveness of CRT was significantly associated with the tumour size. The CR/ CR+PR rates were also significantly associated with the tumour size by logistic regression analysis. There was no significant relationship between histological type and the effectiveness of CRT. In addition, Martel et al. (1997) found stage to be the most important prognostic factors for survival in a similar retrospective study on 76 patients with NSCLC. In our study, the differences of CR/CR+PR rate between the different stages were significant and the former was significantly associated with the stage in the group of patients with CR+PR by logistic regression analysis.

In this study, we evaluated the usefulness of molecular biological markers for the prediction of the sensitivity to CRT in patients with NSCLC. There was a statistically significant correlation between serum levels of CYFRA21-1 before CRT and the effectiveness of the treatment; the CR rates and the effective rates (CR+PR) in CYFRA21-1 high and low groups were significantly different ($p = 0.003$, 0.014, respectively). Thus it showed that NSCLC with a high level of CYFRA21-1 is less sensitive to CRT. Therefore, more attention should be paid to these patients to enhance the sensitivity of CRT. In addition, more precise techniques should be used to promote the volume of the target area, decrease the positioning error and effectively protect the normal tissue. In addition, wider application of a sensitizer for radiotherapy may also be helpful to improve the sensitivity for radiation. However, a larger and more homogeneous sample should be analysed in the future to confirm the promising results of the present work.

Table 5. Relationships between serum levels of CYFRA21-1, neurone-specific enolase (NSE) and carcinoembryonic antigen (CEA) and TNM stage in 127 patients with non-small-cell lung cancer.

		CYFRA21-1 (ng ml^{-1})		CEA (ng ml^{-1})		NSE (ng ml^{-1})	
Variables	*n*	Mean ± SD	*p*-Value	Mean ± SD	*p*-Value	Mean ± SD	*p*-Value
T			0.411		0.144		0.855
T1	23	4.19 ± 2.96		32.10 ± 80.50		33.62 ± 55.32	
T2	37	16.21 ± 56.09		9.50 ± 18.41		29.86 ± 19.45	
T3	38	5.75 ± 7.16		11.40 ± 33.10		32.75 ± 28.92	
T4	29	8.15 ± 10.85		9.97 ± 14.20		37.14 ± 30.95	
N			0.518		0.141		0.332
N0	21	7.25 ± 11.33		14.69 ± 44.04		21.91 ± 9.70	
N1	13	2.91 ± 1.84		4.41 ± 4.06		28.91 ± 15.02	
N2	61	6.56 ± 9.35		8.97 ± 12.06		37.10 ± 31.12	
N3	32	17.51 ± 59.68		28.08 ± 70.28		34.39 ± 48.52	
M			0.868		0.019		0.425
M0	85	6.52 ± 9.89		8.33 ± 14.76		34.73 ± 38.02	
M1	42	6.24 ± 7.33		26.27 ± 66.73		29.70 ± 20.62	

Table 6. Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) of tumour markers in patients with non-small-cell lung cancer.

Tumour markers	Cut-off value (ng ml^{-1})	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CYFRA21-1	4.12	67.09	60.42	74.6	53.6
	3.3	53.16	64.58	71.2	45.6
	5.02	74.68	45.83	69.4	52.4
CEA	3.4	51.9	62.5	69.5	44.1
	5.03	67.09	54.17	70.7	50.0
	5.5	68.35	45.83	67.5	46.8
NSE	17.54	29.1	72.92	63.9	38.5
	23.6	54.43	60.42	69.4	44.6
	25.17	58.23	50.00	65.7	42.1

CEA, carcinoembryonic antigen; NSE, neurone-specific enolase.

Declaration of interest

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References

Ando S, Kimura H, Iwai N, Shima M, Ando M, Kuriyama T. (2001). Optimal combination of seven tumour markers in prediction of advanced stage at first examination of patients with non-small cell lung cancer. *Anticancer Res* 21:3085–92.

Ardizzoni A, Cafferata MA, Tiseo M, Filiberti R, Marroni P, Grossi F, Paganuzzi M. (2006). Decline in serum carcinoembryonic antigen and cytokeratin 19 fragment during chemotherapy predicts objective response and survival in patients with advanced nonsmall cell lung cancer. *Cancer* 107:2842–9.

Buccheri G, Ferrigno D. (1994). Prognostic factors in lung cancer: tables and comments. *Eur Respir J* 7:1350–64.

Cho WC. (2007). Potentially useful biomarkers for the diagnosis, treatment and prognosis of lung cancer. *Biomed Pharmacother* 61:515–19.

Diez M, Torres A, Ortega L, Maestro M, Hernando F, Gomez A, Picardo A, Granell J, Balibrea JL. (1993). Value of serum neuron-specific enolase in nonsmall cell lung cancer. *Oncology* 50:127–31.

Ferrigno D, Buccheri G. (1995). Clinical applications of serum markers for lung cancer. *Respir Med* 89:587–97.

Ferrigno D, Buccheri G, Biggi A. (1994). Serum tumour markers in lung cancer: history, biology and clinical applications. *Eur Respir J* 7:186–97.

Ferrigno D, Buccheri G, Giordano C. (2003). Neuron-specific enolase is an effective tumour marker in non-small cell lung cancer (NSCLC). *Lung Cancer* 41:311–20.

Foa P, Fornier M, Miceli R, Seregni E, Santambrogio L, Nosotti M, Cataldo I, Sala M, Caldiera S, Bombardieri E. (1999). Tumour markers CEA, NSE, SCC, TPA and CYFRA 21.1 in resectable non-small cell lung cancer. *Anticancer Res* 19:3613–18.

Hatzakis KD, Froudarakis ME, Bouros D, Tzanakis N, Karkavitsas N, Siafakas NM. (2002). Prognostic value of serum tumor markers in patients with lung cancer. *Respiration* 69:25–9.

Holdenrieder S, Stieber P, von Pawel J, Raith H, Nagel D, Feldmann K, Seidel D. (2004). Circulating nucleosomes predict the response to chemotherapy in patients with advanced non-small cell lung cancer. *Clin Cancer Res* 10:5981–7.

Holdenrieder S, Stieber P, VON Pawel J, Raith H, Nagel D, Feldmann K, Seidel D. (2006). Early and specific prediction of the therapeutic efficacy in non-small cell lung cancer patients by nucleosomal DNA and cytokeratin-19 fragments. *Ann NY Acad Sci* 1075:244–57.

Hotta K, Segawa Y, Takigawa N, Kishino D, Saeki H, Nakata M, Mandai K, Eguchi K. (2000). Evaluation of the relationship between serum carcinoembryonic antigen level and treatment outcome in surgically resected clinical-stage I patients with non-small-cell lung cancer. *Anticancer Res* 20:2177–80.

Icard P, Regnard JF, Essomba A, Panebianco V, Magdeleinat P, Levasseur P. (1994). Preoperative carcinoembryonic antigen level as a prognostic indicator in resected primary lung cancer. *Ann Thorac Surg* 58:811–14.

Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ. (2006). Cancer statistics, 2006. *CA Cancer J Clin* 56:106–30.

Kulpa J, Wojcik E, Radkowski A, Kolodziejski L, Stasik Z. (2000). CYFRA 21-1, TPA-M, TPS, SCC-Ag and CEA in patients with squamous cell lung cancer and in chemical industry workers as a reference group. *Anticancer Res* 20:5035–40.

Kulpa J, Wojcik E, Reinfuss M, Kolodziejski L. (2002). Carcinoembryonic antigen, squamous cell carcinoma antigen, CYFRA 21-1, and neuronspecific enolase in squamous cell lung cancer patients. *Clin Chem* 48:1931–7.

Lamy P, Grenier J, Kramar A, Pujol JL. (2000). Pro-gastrin-releasing peptide, neuron specific enolase and chromogranin A as

serum markers of small cell lung cancer. *Lung Cancer* 29: 197–203.

Maria WW, Ying X, Edward P, Walter JC, Walter H. (2001). Assessment of lung cancer response after nonoperative therapy: tumor diameter, bidimensional product, and volume. A serial CT scan-based study. *Int J Radiat Oncol Biol Phys* 51:56–61.

Maria WW, Suzanne S, Jeffrey B, Mary G, Bahman E, James P, William S. (2008). Increasing tumor volume is predictive of poor overall and progression –free survival: secondary analysis of the radiation therapy oncology group 93-11 phase I-II radiation dose-escalation study in patients with inoperable non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys* 70:385–90.

Martel MK, Strawderman M, Hazuka MB, et al. (1997). Volume and dose parameters for survival of non-small cell lung cancer patients. *Radiother Oncol* 44:23–9.

Molina R, Filella X, Augé JM, Fuentes R, Bover I, Rifa J, Moreno V, Canals E, Violas N, Marquez A, Barreiro E, Borras J, Viladiu P. (2003). Tumor markers (CEA, CA 125, CYFRA 21-1, SCC and NSE) in patients with non-small cell lung cancer as an aid in histological diagnosis and prognosis. Comparison with the main clinical and pathological prognostic factors. *Tumour Biol* 24:209–18.

Molina R, Auge JM, Filella X, Violas N, Alicarte J, Domingo JM, Ballesta AM. (2005). Pro-gastrin-releasing peptide (proGRP) in patients with benign and malignant diseases: comparison with CEA, SCC, CYFRA 21-1 and NSE in patients with lung cancer. *Anticancer Res* 25(3A):1773–8.

Niklinski J, Furman M, Chyczewska E, Chyczewski L, Rogowski F, Laudanski J. (1995). Diagnostic and prognostic value of the new tumour marker CYFRA 21-1 in patients with squamous cell lung cancer. *Eur Respir J* 8:291–4.

Nisman B, Lafair J, Heching N, Lyass O, Baras M, Peretz T, Barak V. (1998). Evaluation of tissue polypeptide specific antigen, CYFRA 21-1, and carcinoembryonic antigen in nonsmall cell lung carcinoma: does the combined use of cytokeratin markers give any additional information? *Cancer* 82:1850–9.

Okada M, Nishio W, Sakamoto T, Uchino K, Yuki T, Nakagawa A, Tsubota N. (2004). Prognostic significance of perioperative serum carcinoembryonic antigen in non-small cell lung cancer: analysis of 1,000 consecutive resections for clinical stage I disease. *Ann Thorac Surg* 78:216–21.

Pujol JL, Grenier J, Daurès JP, Daver A, Pujol H, Michel FB. (1993). Serum fragment of cytokeratin subunit 19 measured by CYFRA 21-1 immunoradiometric assay as a marker of lung cancer. *Cancer Res* 53:61–6.

Pujol JL, Molinier O, Ebert W, Daurès JP, Barlesi F, Buccheri G, Paesmans M, Quoix E, Moro-Sibilot D, Szturmowicz M, Bréchot JM, Muley T, Grenier J. (2004). CYFRA 21-1 is a prognostic determinant in non-small-cell lung cancer: results of a meta-analysis in 2063 patients. *Br J Cancer* 90:2097–105.

Ray P, Quantin X, Grenìer J, Pujol JL. (1998). Predictive factors of tumor response and prognostic factors of survival during lung cancer chemotherapy. *Cancer Detect Prev* 22:293–304.

Reinmuth N, Brandt B, Semik M, Kunze WP, Achatzy R, Scheld HH, Broermann P, Berdel WE, Macha HN, Thomas M. (2002). Prognostic impact of CYFRA21-1 and other serum markers in completely resected non-small cell lung cancer. *Lung Cancer* 36: 265–70.

Schneider J, Philipp M, Velcovsky HG, Morr H, Katz N. (2003). Pro-gastrin-releasing peptide (ProGRP), neuron specific enolase (NSE), carcinoembryonic antigen (CEA) and cytokeratin 19-fragments (CYFRA 21-1) in patients with lung cancer in comparison to other lung diseases. *Anticancer Res* 23:885–93.

Segawa Y, Ohnoshi T, Hiraki S, Ueoka H, Kiura K, Kamei H, Tabata M, Shibayama T, Miyatake K, Genba K. (1993). Immunohistochemical detection of P-glycoprotein and carcinoembryonic antigen in small cell lung cancer: with reference to predictability of response to chemotherapy. *Acta Med Okayama* 47:181–9.

Trani L, Myerson J, Ashley S, Young K, Sheri A, Hubner R, Puglisi M, Popat S, O'Brien ME. (2010). Histology classification is not a predictor of clinical outcomes in advanced non-small cell lung cancer (NSCLC) treated with vinorelbine or gemcitabine combinations. *Lung Cancer* March 12. [Epub ahead of print].

Vansteenkiste J, Fischer BM, Dooms C, Mortensen J. (2004). Positron-emission tomography in prognostic and therapeutic assessment of lung cancer: systematic review. *Lancet Oncol* 5:531–40.

Vollmer RT, Govindan R, Graziano SL, Gamble G, Garst J, Kelley MJ, Christenson RH. (2003). Serum CYFRA 21-1 in advanced stage non-small cell lung cancer: an early measure of response. *Clin Cancer Res* 9:1728–33.

Werner-Wasik M, Xiao Y, Pequignot E, CurranWJ, HauckW. (2001). Assessment of lung cancer response after nonoperative therapy: tumor diameter, bidimensional product, and volume. A serial CT scan-based study. *Int J Radiat Oncol Biol Phys* 51: 56–61.