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Prognostic value of combination of Cyfra 21-1, CEA and NSE in patients with advanced non-small cell lung cancer

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Summary *Objective*: To assess the value of Cyfra 21-1, carcino-embryonic antigen (CEA) and neuron-specific enolase (NSE) combined, all three together as prognostic factors in advanced stage non-small cell lung cancer (NSCLC) patients.

Patients and methods: Serum samples from untreated NSCLC patients were prospectively collected. All assays were performed using commercial kits blind to clinical information. Serum levels of CEA, NSE and Cyfra 21-1 higher than 10, 13 and 3.5 ng/ml, respectively, were considered as elevated.

Results: 264 patients (men, 87%), with Performans Status (PS) of 0/1 in 80% and stage IV disease in 65% were studied. Cyfra 21-1, CEA and NSE were elevated in 52.5%, 41.8% and 33.2% of patients, respectively. Median survival was 9 months (range, 1–77). Cyfra 21-1, age, PS, stage as well as the combination of the three markers together correlated with prognosis in univariate analysis. Multivariate analysis demonstrated that age $\geqslant$65 years (HR = 1.3 [1.02–1.70], $p = 0.03$), PS 2 (HR = 4.3 [3.13–6.11], $p < 0.0001$), Cyfra 21-1 $\geqslant$ 3.5 ng/ml (HR = 1.3 [1.06–1.78], $p = 0.01$) and the combination of the three markers (HR = 1.06 [1.009–1.13], $p = 0.02$) remained prognostic determinants.

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Conclusion: Combining Cyfra 21-1, NSE and CEA correlated with prognosis in a significant and independent manner.

Introduction

Lung cancer is the major cause of cancer-related death in western countries. Research on prognostic factors in non-small cell lung cancer (NSCLC) is of great importance because it potentially leads to a better and perhaps tailored management of patients. Some serum markers are potential prognostic factors in NSCLC patients. However, serum markers are interesting if they give a supplementary and independent information on prognosis which is currently principally based on Performans Status (PS) and TNM classification.[1] Increased level of a recognized and independent marker should be able to modify the therapeutic strategy as for nodal or metastatic status for example. None of the markers is actually considered when deciding treatment option.

Among these NSCLC markers Cyfra 21-1, a fragment of cytokeratin subunit 19, is of great interest. Indeed, Cyfra 21-1 was found in several studies to be correlated with TNM stage and PS and reflecting prognosis of NSCLC patients in an independent manner.[2–7] Then, Pujol and others suggested that Cyfra 21-1 should be regarded as a co-variable in future NSCLC trials.[2]

Neuron-specific enolase (NSE) was evaluated in NSCLC and high NSE level was found to be of poor prognosis.[8] High NSE level could reflect a neuroendocrine component in NSCLC leading to better response to chemotherapy[9] but poorest outcome. However, prognostic implication of NSE in NSCLC patients management has to be clearly specified.

Carcino-embryonic antigen (CEA) is currently not considered in prognostic evaluation of NSCLC patients given the results of previous studies.[10,11] Nevertheless, discordance persists between authors, and CEA could be useful under particular conditions such as response evaluation or resected patients.[12,13]

In this study, we hypothesize that combination of Cyfra 21-1, CEA and NSE considered together could have a better prognostic value in NSCLC than each of them considered separately.

Patients and methods

Patients

Serum samples from untreated lung cancer patients were prospectively collected from January 1994 to December 2000. Eligibility criteria consisted of histologically or cytologically proven NSCLC. Patients suffering from small cell lung cancer were not eligible. Histological subclassification was done according to the World Health Organization classification.[14] PS was estimated using the Eastern Cooperative Oncology Group scale. Clinical examination, chest, abdomen and brain computed tomographic scan, bronchoscopy and bone scanning were carried out systematically. Staging was done upon these procedures according to the TNM classification.[15]

Treatments

A medical panel composed of thoracic surgeons, chest physicians and radiotherapists examined each patient's record. Treatments were decided according to the standard of care. Stage IIIB patients were generally treated using an association of sequential chemotherapy (cisplatin and etoposide) and thoracic radiotherapy (60 grays in 30 fractions), while stage IV patients received platinum-based chemotherapy (with vinorelbine, gemcitabine or paclitaxel) for a maximum of six cycles.

Biochemical measurements

Serum samples were obtained from each patient before initiation of treatment. Fresh serum was collected and cooled after sampling, than stored at −20°C until dosage. All assays were performed using commercial kits (ELSA 2 CEA CisBiointernational™; ELSA Cyfra 21-1 CisBiointernational™; NSE-Ria Pharmacia™) blind to clinical information.

Serum levels of CEA, NSE and Cyfra 21-1 were considered as elevated when they were superior or equal to 10, 13 and 3.5 ng/ml, respectively. Cut-off value of 10 ng/ml for CEA was determined according to our previous findings.[12] Cut-off values of 3.5 ng/ml for Cyfra 21-1 and 13 ng/ml for NSE were based on previously published results.[2,8,9]

Statistics

Survival data were updated in July 2003. One patient was lost. Probability of survival was estimated using the Kaplan–Meier method. Differences between survival were tested by means of log-rank test. A multivariate regression analysis

was done with Cox's regression using the forward maximum likelihood method. All variables with a p-value less than 0.20 at the time of univariate analysis were entered into the model. A p-value less than 0.05 was considered as significant.

Results

Patients characteristics

Overall, 264 patients with a median age of 62 years (range, 27–85 years) were studied. Demographic data and clinical characteristics of patients are summarized in Table 1. One hundred and eleven patients (41.8%) had serum level of CEA >10 ng/ml, 87 (33.2%) had NSE level >13 ng/ml and 138 (52.5%) had Cyfra 21-1 level >3.5 ng/ml. Table 2 summarize the results for the various combinations of all the three serum markers measurements.

Survival analysis

Survival was analyzed in the whole patient population. Median follow-up was 9 months [range, 1–77 months]. There were 261 events and median survival was 9 months [range, 1–77]. A significant difference ($p=0.0001$) was seen between the seven categories. In univariate analysis, age (<65 versus $\geqslant 65$ years, $p=0.01$), PS (0/1 versus 2, $p<0.00001$) and TNM (stage IIIB versus IV, $p=0.01$) showed statistically significant influence on prognosis. Serum level of Cyfra 21-1 (<3.5 versus $\geqslant 3.5$ ng/ml, $p=0.0001$) considered alone or

Table 1 Patient's characteristics.

		Patients	%
Gender	Men/Women	230/34	87/13
Age (years)	$\leqslant 65/>65$	163/101	62/38
Histology	ADK	90	34
	SCC	118	45
	LCC	56	21
PS	0/1	129/83	49/31
	2	52	20
Stage	IIIB	92	35
	IV	172	65

ADK, adenocarcinoma; SCC, squamous cell carcinoma; LCC, large cell carcinoma; and PS, performans status.

Table 2 Results for the various combinations of all the three serum markers measurements.

Marker			Patients	
Cyfra 21-1 (ng/ml)	CEA (ng/ml)	NSE (ng/ml)	n	%
—	—	—	49	18.5
>3.2	—	—	62	23.5
>3.2	>10	—	26	9.8
>3.2	—	>13	15	6
—	>10	—	40	15
—	>10	>13	10	3.8
—	—	>13	27	10.2
>3.2	>10	>13	35	13.2

ADK, adenocarcinoma; SCC, squamous cell carcinoma; and LCC, large cell carcinoma.

Table 3 Results of univariate analysis.

Variable		Survival months (median)	p (log-rank)
Gender	Men/Women	7/9	0.811
Age (years)	$\leqslant 65/>65$	9/8	0.011
PS	0/1	10	$< 10^{-5}$
	2	2	
Stage	IIIB	11	0.01
	IV	7	
Serum marker (ng/ml)	CEA >10	7	0.13
	CEA <10	10	
	NSE >13	7	0.081
	NSE <13	10	
	Cyfra 21-1 >3.5	7	0.0001
	Cyfra 21-1 <3.5	12	

ADK, adenocarcinoma; SCC, squamous cell carcinoma; LCC, large cell carcinoma; PS, performans status; CEA, carcinoembryonic antigen; and NSE, neuron-specific enolase

Table 4 Univariate analysis, results for the various combinations of all the three serum markers measurements.

Marker			Survival	
Cyfra 21-1 (ng/ml)	CEA (ng/ml)	NSE (ng/ml)	Median (months)	
—	—	—	11	
>3.2	—	—	10	
>3.2	>10	—	5	
>3.2	—	>13	4	$p = 0.0001$
—	>10	—	13	
—	>10	>13	15	
—	—	>13	10	
>3.2	>10	>13	3	

CEA, carcino-embryonic antigen; and NSE, neuron-specific enolase.

Table 5 Results of multivariate analysis.

Variable	HR	*p* value	95% CI
Age ⩽65/>65 years	1.3	0.03	1.02–1.70
PS 0-1/2	4.3	<0.0001	3.13–6.11
TNM IIIB/IV	—	Ns	—
Cyfra 21-1 <3.5/⩾3.5	1.3	0.016	1.06–1.78
CEA <10/⩾10	—	Ns	—
NSE <13/⩾13	—	Ns	—
Combination of the three markers	1.06	0.02	1.009–1.13

PS, performans status; CEA, carcino-embryonic antigen; and NSE, neuron-specific enolase.

combined with CEA and NSE ($p = 0.0001$) also showed statistically significant influence on prognosis. Overall results of univariate survival analysis are summarized in Tables 3 and 4. Multivariate analysis using Cox's model for the 264 patients demonstrated that poor PS (i.e. 0/1 versus 2, $p < 0.0001$), age older than 65 years ($p = 0.03$), level of Cyfra 21-1 higher than 3.5 ng/ml ($p = 0.01$) and the combination of the three serum tumor markers considered together ($p = 0.02$) remained prognostic determinants (Table 5).

Discussion

Several serum markers were tested in NSCLC patients and proved to be of low value. Indeed, only Cyfra 21-1 clearly independently predicts survival. In this study, we hypothesize that combination of Cyfra 21-1, CEA and NSE considered together could have a better prognostic value than each of them considered separately. We showed differences in survival between patients with normal and high level of Cyfra 21-1 ($p = 0.0001$) but not CEA or NSE considered alone. Considered all three together, Cyfra 21-1, CEA and NSE highly correlated with survival in univariate analysis ($p = 0.0001$). Furthermore, Cyfra 21-1 reached statistical significance in multivariate analysis ($p = 0.01$) as well as the combination of the three tumor markers considered together ($p = 0.02$). Considering the three markers together, adding NSE and CEA to Cyfra 21-1 demonstrated an independent value in prognostic evaluation.

A significant additive value of CEA and Cyfra 21-1 in lung adenocarcinoma was showed by Ando and colleagues.[17] Indeed, high levels of both these markers were correlated with advanced stage of disease. However, prognostic significance of CEA is discussed for a long time with conflicting results. Indeed, some studies showed a negative prognostic value for CEA[7,18] while others did not.[19–21] These discrepancies probably highlight the need for specific evaluation of CEA in distinct subgroups according to histology[22] or stage of disease.[17]

Prognostic value of high NSE level did not reach statistical significance neither in univariate ($p = 0.08$) nor in multivariate analysis. This was in discrepancy with previous studies.[3,8,9] Indeed, we applied a cut-off value of 13 ng/ml in accordance with previously published study.[3,8,9] However, a better specificity for NSCLC could be obtained with a cut-off value of 20 ng/ml[16] but decreasing

sensibility (i.e. the number of patients with high NSE level). Then, the choice of the most suitable cut-off value for NSE in the setting of NSCLC has to be discussed. Prognostic value of Cyfra 21-1 was confirmed with a significant relation with survival in multivariate analysis as reported before.[2–6] Moreover, we confirmed the optimal cut-off of 3.5 ng/ml value for Cyfra 21-1 in prognostic evaluation.[2,3]

Grouped analysis of different markers, each associated with a possible prognostic value, should be again investigated because potentially improving prognostic information[23] and use of mathematical tools in this setting could be helpful and more powerful.[24] Regarding our results, more studies of several blood molecules as prognostic markers are needed including previously proposed biological markers such as lactate dehydrogenase, alkaline phosphatase, albumin, serum sodium, hemoglobin and leukocytes in the aim to select the most suitable co-variables for clinical trials.[25] Nevertheless, despite their independent prognostic value, neither Cyfra 21-1 nor the combination of Cyfra 21-1 with CEA and NSE actually influence the therapeutic strategy. However, their impact on the assessment of the efficacy of the chemotherapy was demonstrated.[26,27] Indeed, the decrease of Cyfra 21-1 level after one cycle of chemotherapy could predict the clinical and radiological response. In this way, initial serum markers level is significantly correlated with survival and its evolution could lead to the chemotherapy regimen adaptation. Then, more studies in this setting are needed.

References

1. Brundage MD, Davies D, Mackillop WJ. Prognostic factors in non-small cell lung cancer: a decade of progress. *Chest* 2002;**122**:1037–57.
2. Reinmuth N, Brandt B, Semik M, et al. Prognostic impact of Cyfra 21-1 and other serum markers in completely resected non-small cell lung cancer. *Lung Cancer* 2002;**36**:265–70.
3. Pujol JL, Boher JM, Grenier J, Quantin X. Cyfra 21-1, neuron specific enolase and prognosis of non-small cell lung cancer: prospective study in 621 patients. *Lung Cancer* 2001;**31**: 221–31.
4. Hirashima T, Takada M, Komiya T, et al. Prognostic significance of CYFRA 21-1 in non-small cell lung cancer. *Anticancer Res* 1998;**18**:4713–6.
5. Niklinski J, Burzykowski T, Niklinska W, et al. Preoperative CYFRA 21-1 level as a prognostic indicator in resected nonsmall cell lung cancer. *Eur Respir J* 1998;**12**:1424–8.
6. Nisman B, Lafair J, Heching N, et al. 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* 1998;**82**:1850–9.
7. Moro D, Villemain D, Vuillez JP, Delord CA, Brambilla C. CEA, CYFRA 21-1 and SCC in non-small cell lung cancer. *Lung Cancer* 1995;**13**:169–76.
8. Diez M, Torres A, Ortega L, et al. Value of serum neuron-specific enolase in nonsmall cell lung cancer. *Oncology* 1993; **50**:127–31.
9. Van Zandwijk N, Jassem E, Bonfrer JMG, Mooi WJ, van Tinteren H. Serum neuro-specific enolase and lactate dehydrogenase as predictors of response to chemotherapy and survival in non-small cell lung cancer. *Semin Oncol* 1992;**19**:37–43.
10. Société de Pneumologie de Langue Française. Recommandations concernant l'utilisation des marqueurs tumoraux sériques dans la prise en charge des cancers bronchiques primitifs. *Rev Mal Respir* 1997;**14**:3S5–9.
11. American Thoracic Society and European Respiratory Society. Pretreatment evaluation of non-small cell lung cancer. *Am Rev Respir Crit Care Med* 1997;**156**:320–32.
12. Kleisbauer JP, Castelnau O, Thomas P, Ramirez J, Lanteaume A, Roux F. Prognostic value of pre-therapeutic levels of carcino-embryonic antigen in primary bronchial carcinoma. *Bull Cancer* 1995;**82**:1019–24.
13. Sawabata N, Ohta M, Takeda S, et al. Serum carcinoembryonic antigen level in surgically resected clinical stage I patients with non-small cell lung cancer. *Ann Thorac Surg.* 2002;**74**:174–9.
14. Brambilla E, Travis WD, Colby TV, Corrin B, Shimosato Y. The new World Health Organization classification of lung tumours. *Eur Respir J* 2001;**18**:1059–68.
15. Moutain CF. Revisions in the international system for staging lung cancer. *Chest* 1997;**111**:1710–7.
16. Satoh H, Ishikawa H, Kurishima K, Yamashita YT, Ohtsuka M, Sekizawa K. Cut-off levels of NSE to differentiate SCLC from NSCLC. *Oncol Rep* 2002;**9**:581–3.
17. Ando S, Kimura H, Iwai N, Shima M, Ando M, Kuriyama T. Optimal combination of seven tumour markers in prediction of advanced stage at first examination of patients with non-small cell lung cancer. *Anticancer Res* 2001;**21**: 3085–92.
18. Rubins JB, Dunitz J, Rubins HB, Maddaus MA, Niewoehner DE. Serum carcinoembryonic antigen as an adjunct to preoperative staging of lung cancer. *J Thorac Cardiovasc Surg* 1998;**116**:412–6.
19. Margolis ML, Hyzy JB, Schenken LL, Schepart BS. Serum tumor markers in non-small cell lung cancer. A comparative analysis. *Cancer.* 1994;**73**:605–9.
20. Kulpa J, Wojcik E, Radkowski A, Kolodziejski L, Stasik Z. 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* 2000;**20**: 5035–40.
21. Hatzakis KD, Froudarakis ME, Bouros D, Tzanakis N, Karkavitsas N, Siafakas NM. Prognostic value of serum tumor markers in patients with lung cancer. *Respiration* 2002;**69**: 25–9.
22. Pelletier MP, Edwardes MD, Michel RP, Halwani F, Morin JE. Prognostic markers in resectable non-small cell lung cancer: a multivariate analysis. *Can J Surg* 2001;**44**: 180–8.
23. Nisman B, Lafair J, Heching N, et al. 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* 1998;**82**:1850–9.
24. Schneider J, Bitterlich N, Velcovsky HG, Morr H, Katz N, Eigenbrodt E. Fuzzy logic-based tumor-marker profiles

improved sensitivity in the diagnosis of lung cancer. *Int J Clin Oncol* 2002;**7**:145–51.
25. Watine J. Serum Cyfra 21-1 as a co-variable in future NSCLC chemotherapy trials. *Lung Cancer* 2001;**33**:89–91.
26. Vollmer RT, Govindan R, Graziano SL, et al. Serum CYFRA 21-1 in advanced stage non-small cell lung cancer: an early measure of response. *Clin Cancer Res.* 2003; **9**:1728–33.
27. Ebert W, Muley T. CYFRA 21-1 in the follow-up of inoperable non-small cell lung cancer patients treated with chemotherapy. *Anticancer Res.* 1999;**19**:2669–72.