Pergamon

European Journal of Cancer Vol. 30A, No. 5, pp. 601–606, 1994

0959–8049/94 $7.00+0.00

0959-8049(94)E0008-R

CYFRA 21-1, a Sensitive and Specific New Tumour Marker for Squamous Cell Lung Cancer. Report of the First European Multicentre Evaluation

D. Rastel, A. Ramaioli, F. Cornillie and B. Thirion on behalf of the CYFRA 21-1 Multicentre Study Group

The present study was designed to determine whether CYFRA 21-1, measuring cytokeratin 19, could be a specific and sensitive tumour marker for non-small cell lung cancer (NSCLC). Serum measurements were made at diagnosis in 2250 patient samples by an immunoradiometric "sandwich type" assay, using two cytokeratin 19 specific monoclonal antibodies. Among healthy individuals (n = 711) and patients with benign lung disease (n = 546), 95 percentiles were 1.2 and 2.95 ng/ml, respectively. Cumulative distribution analysis curves were established. From these data, 3.3 ng/ml gave 96% specificity. Using this cutoff, the sensitivity for small cell lung cancer was 16% (n = 74) compared to 41% for NSCLC (n = 547). In histological sub-groups, sensitivity was 57% for squamous cell lung cancer, 34% for undifferentiated large cell carcinoma and 27% for adenocarcinoma. the level of CYFRA 21-1 was correlated with tumour size and UICC stage. In squamous cell lung cancer, the sensitivity of the squamous cell carcinoma marker was 30%, 25% for carcinoembryonic antigen and 46% for tissue polypeptide antigen, using the same series of samples and cutoffs defined at 96% specificity. In conclusion, CYFRA 21-1 is a sensitive tumour marker for NSCLC, especially squamous cell lung cancer.

Key words: tumour markers, lung neoplasms, cytokeratin 19, radioimmunoassay, carcinoembryonic antigen, squamous cell carcinoma

Eur J Cancer, Vol. 30A, No. 5, pp. 601–606, 1994

INTRODUCTION

THE CYTOSKELETON is a complex filamentous cytoplasmic structure that may influence dynamic cell morphology in the tissue environment [1]. It is composed of different types of filaments with different sizes: actin, intermediate filaments, myosine and microtubules. Cytokeratins are the major component of intermediate filaments [2]. The different polypeptides of this complex family can be separated by their electrophoretic migration pattern into types I (acidic) and II (basic) or by their molecular weights of 20 to 68 kDa. At present, 20 cytokeratins have been described, and all are proteins restricted only to epithelial cells [3, 4]. Cellular cytokeratins are made up of heterodimers, each containing one type I and one type II cytokeratin filament. This cytokeratin pattern is tissue specific and varies with the cell differentiation [5].

Cytokeratin 19 (Ck19; type I –40 kDa) is only expressed in simple epithelia such as the unstratified or pseudo-stratified epithelium of the bronchial tree. Immunohistochemical studies using LP2K and BA 17 Ck 19 specific monoclonal antibodies revealed Ck 19 expression in normal lung epithelium, and enhanced expression in malignant lung tissues. The cytokeratin pattern is well preserved during the transformation from normal to malignant tissue [6]. Thus, cytokeratins may be helpful in classifying lung tumours. Ck 19 is released into the bloodstream upon cell death and, therefore, it may be used as a serum tumour marker.

Lung cancer is a major medical problem as its incidence is steadily increasing. According to the WHO classification system primary lung cancers are divided into four major histological types: small cell carcinoma, squamous cell carcinoma, adenocarcinoma and undifferentiated large cell carcinoma [7]. Although recent studies demonstrated a common origin of malignant cell lines, the clinical pattern of the disease separates small cell lung cancers (SCLC) from the other group of non-small cell lung cancers (NSCLC). Compared to SCLC, the NSCLC may remain localised for longer, and is somewhat less aggressive. Hence, surgical treatment remains possible for some NSCLC, sometimes leading to a better survival. Progression to advanced metastatic disease is the most frequent cause of treatment failure and relapse during follow-up of these patients. Therefore, accurate investigations of patients in the follow-up should be repeated regularly, and a reliable tumour marker would be very

Correspondence to D. Rastel.
D. Rastel and B. Thirion are at the CIS bio International, BP 32, 91192 Gif sur Yvette; A. Ramaioli is at the Unité de Statistiques Médicales, Centre Antoine Lacassagne, 36 Voie Romaine, 06054 Nice cedex, France; and F. Cornillie is at the Centocor Diagnostics Europe, Desguinlei 50, 2018 Antwerp, Belgium.
Revised 29 Dec. 1993, accepted 5 Jan. 1994.

EJC 30:5-C

helpful to assist in making therapy decisions and to detect relapses earlier [8].

Several tumour markers have been evaluated in SCLC: neuron specific enolase (NSE) [9], also in NSCLC; carcinoembryonic antigen (CEA); squamous cell carcinoma (SCC); tissue polypeptide antigen (TPA); and alpha feto protein (AFP) [8, 10–12]. Some studies indicate a prognosis value with the ability to monitor the efficacy of treatment and the usefulness in follow-up of patients for NSE, CEA and TPA to some extent.

In this study, we investigated the sensitivity and specificity of CYFRA 21-1 (Centocur Diagnostics, Malvern, Pennsylvania, U.S.A.), a new tumour marker measuring Ck 19 fragments in serum. CYFRA 21-1 results were compared to those obtained with other tumour markers sometimes used for NSCLC, such as CEA, SCC and TPA.

PATIENTS AND METHODS

Sampling and biochemical methods

Two thousand two hundred and fifty patient sera, tested at 11 sites in five different European countries, were included in this retrospective study. Nine centres provided patients for all study groups, i.e. healthy controls, benign non-lung diseases, benign lung diseases and malignant diseases including lung cancers. Blood samples were collected at the time of diagnosis before any treatment, and serum was collected freshly after sampling. Sera were stored at −20°C. Sera were assayed using a simultaneous immunoradiometric sandwich type assay using two monoclonal antibodies: KS 19-1 and BM 19-21 reactive with different epitopes on Ck 19 (CYFRA 21-1).

Controls

Seven hundred and eleven healthy blood donors were included: 489 males and 222 females, their age ranged from 18 to 79 years; the mean age was 41 years.

Patients

Benign lung diseases (n = 546; mean age 57 years) were divided in chronic obstructive pneumopathies (n = 112), acute infectious diseases (n = 94), tuberculosis (n = 96), asthma (n = 74), diffuse non-infectious interstitial diseases (n = 130) and others (n = 40) with a ratio of 37.5% infectious and 62.5% non-infectious diseases.

Patients of both sexes with a histologically proven and previously untreated primary lung cancer were entered into the study. Cancers were divided in SCLC (n = 74; mean age 60 years) and NSCLC (n = 547; mean age 59 years). NSCLC were separated according to the WHO classification [7] into squamous cell carcinoma (n = 277), adenocarcinoma (n = 172) and undifferentiated large cell carcinoma (n = 98).

Histological classification was only conducted on biopsy specimens. Staging was performed using the 4th edition of the UICC TNM classification and the American Thoracic Society map of regional pulmonary nodes [13, 14].

Statistics

Distribution of CYFRA 21-1 values in serum was described by means of median, range and percentiles since CYFRA 21-1 values did not reveal Gaussian distribution [15]. Sensitivity and specificity patterns were studied by cumulative distribution analysis (CDA) [16]. Differences between two independent groups were determined by Mann–Whitney U test; differences between more than two groups were determined by means of Kruskal–Wallis one-way analysis of variance. A probability (P) level < 0.05 was considered as significant. Proportions of elevated serum CYFRA 21-1 levels in subgroups were compared by χ^2 test with Yates' correction when appropriate. Comparisons between markers were made using a 96% specificity versus the non-malignant lung disease population for all markers (n = 546). At 96% specificity, the cutoff values for CEA (CIS bio International), TPA (Byk Sangtec) and SCC (Abbott) were 7.5 ng/ml, 170 U/l and 2.5 ng/ml, respectively. Further comparisons between CYFRA 21-1 and TPA were made using receiver operating characteristic (ROC) curves [17]. The area under the curve was calculated for CYFRA 21-1 and TPA [18]. CDA and ROC curves are rather similar simultaneous representations of sensitivity and specificity; the CDA curve indicates the cutoff value, whereas the ROC curve is easier to read for direct comparison of tumour markers but without indication of the cutoff.

RESULTS

Controls

In 711 healthy patients, CYFRA 21-1 serum assay values ranged from 0 to 3.5 ng/ml, the median was 0.4 ng/ml; 95th and 99th centiles were 1.2 and 1.95 ng/ml, respectively. No significant differences were found between male and female populations (P = 0.42), smokers (n = 439) and non-smokers (n = 164; P = 0.95), and 5-years age groups (P = 0.12) using the Mann–Whitney and Kruskal–Wallis tests.

Benign lung diseases

In benign lung diseases (n = 546), CYFRA 21-1 serum assay values ranged from 0.01 to 18.00 ng/ml, the median was 0.5 ng/ml. No significant differences were found between benign diseases groups (P = 0.08). A serum level of 3.3 ng/ml indicated 96% specificity of CYFRA 21-1 in benign lung diseases (Figure 1).

Non-lung benign diseases

Sensitivity of CYFRA 21-1 was also studied in non-lung benign diseases (Table 1). At a cutoff of 3.3 ng/ml, the specificity was 100% in gynaecological diseases (ovarian cysts, endometriosis, gynaecological tract infections), 100% in colorectal and gastric benign diseases, 100% in acute hepatitis, 88% in cirrhosis and 67% in chronic renal failure.

Lung cancers

Sensitivity of CYFRA 21-1 in SCLC and NSCLC samples is shown in Figure 2. Using the cutoff of 3.3 ng/ml (96% of specificity for benign lung diseases), the sensitivity of CYFRA 21-1 was 16% for SCLC and 41% for NSCLC. The CDA curve shows that at 90% specificity for benign lung diseases (2 ng/ml), the CYFRA 21-1 sensitivity was 55% in NSCLC and 32.5% in SCLC.

Further analyses were calculated only in the NSCLC group. In NSCLC patients, CDA curves were plotted for the different histological subtypes (Figure 3). Best specificity and sensitivity was obtained at 3.3 ng/ml. At this cutoff, the sensitivity of CYFRA 21-1 in squamous cell carcinoma, adenocarcinoma and undifferentiated large cell carcinoma was 56.7, 27.3 and 33.7%, respectively.

CYFRA 21-1 and TNM classification

We also measured CYFRA 21-1 assay values in different groups according to the TNM classification. In order to evaluate the relationship of marker values to the tumour mass, only

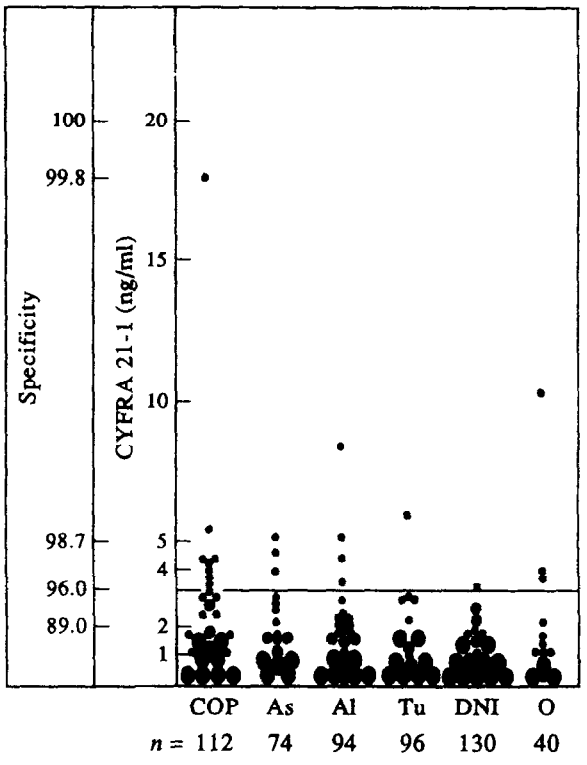

Figure 1. Assay values (ng/ml) and specificity (%) of CYFRA 21-1 in benign lung diseases. COP, chronic obstructive bronchopneumopathy; As, asthma; Al, acute infectious diseases; Tu, tuberculosis; DNI, diffuse interstitial non-infectious diseases; O, other benign lung diseases. ◯ 10 subjects, ○ 5 subjects, ◦ 1 subject.

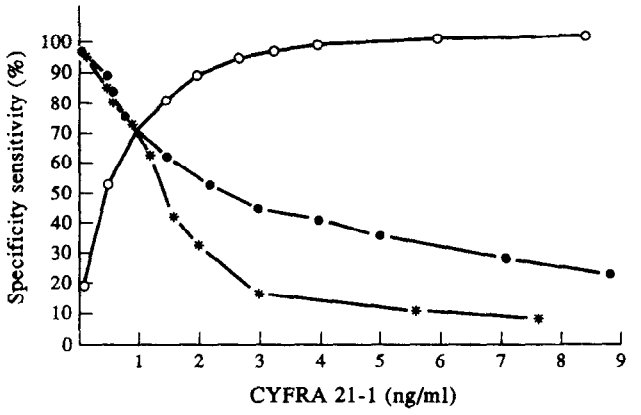

Figure 2. Cumulative distribution analysis curves of the small cell lung cancers (SCLC) and non-small cell lung cancers (NSCLC) compared with benign lung diseases. *——* SCLC, $n = 74$; ●——● NSCLC, $n = 547$; ○——○ benign lung diseases, $n = 546$.

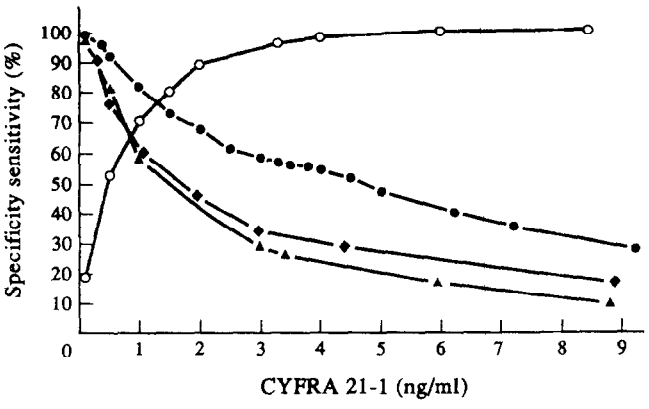

Figure 3. Cumulative distribution analysis curves of squamous cell lung carcinoma (●——●; $n = 277$), adenocarcinoma (▲——▲; $n = 172$) and undifferentiated large cell carcinoma (◆——◆; $n = 98$). Specificity is based on the non-malignant lung disease group (○——○; $n = 546$).

N0M0 or N1M0 NSCLC were studied ($n = 153$). There was an increase of CYFRA 21-1 serum assay values from T1 to T3, with a decrease in T4, although the T of the TNM classification from T3 to T4 is not considered to fit completely with the tumour size. The median of CYFRA 21-1 values was different between the groups studied ($P < 0.05$) (Table 2). The difference between CYFRA 21-1 assay values for groups T1T2N0N1 ($n = 113$; median 1.3 ng/ml) and T3T4N2N3 ($n = 290$; median 4.4 ng/ml) was significant ($P < 0.0001$, Mann–Whitney test). It has been suggested recently that the first group contains operable patients, whereas the second is composed of inoperable patients.

In squamous cell carcinoma, the sensitivity of CYFRA 21-1 was 38% in stages I and II; 43% in stage IIIa; 79% in stage IIIb and 69% in stage IV (Figure 4).

Knowing that the separation between stages I, II, IIIa ($n = 122$) and stages IIIb, IV ($n = 106$) is generally recognised as a criteria on which surgeons are used to basing their surgical decision, we grouped the CYFRA 21-1 values accordingly to evaluate the sensitivity in these two groups of patients. At 13 ng/ml, the positive predictive value of CYFRA 21-1 was 78% and the negative predictive value was 64%.

Table 1. Distribution of CYFRA 21-1 serum values in benign diseases

Other non-malignant diseases	*n*	Median	Range	>3.3 ng/ml	Specificity (%)
Digestive (without liver)	16	0.29	0.05–1.58	0	100
Liver diseases	86	0.73	0.05–6.90	5	94
Hepatitis	43	0.70	0.05–3.20	0	100
Cirrhosis	43	1.20	0.05–6.90	5	88
Gynaecological diseases	21	0.11	0.05–0.93	0	100
Chronic renal failure	63	2.63	0.05–7.00	21	67
Total	186	1.00	0.05–7.00	26	86
Total (without chronic renal failure)	123	0.70	0.05–6.90	5	96

Units are expressed in ng/ml.

Table 2. Comparison of CYFRA 21-1 values in N0N1M0 group of NSCLC based on "T" differences of the TNM classification by means of the Kruskal–Wallis test

N0N1M0	n	CYFRA 21-1 median (ng/ml)	Difference between groups
T1	42	0.78	
T2	71	1.80	Kruskal and Wallis test
T3	24	2.25	$P<0.05$
T4	16	1.32	

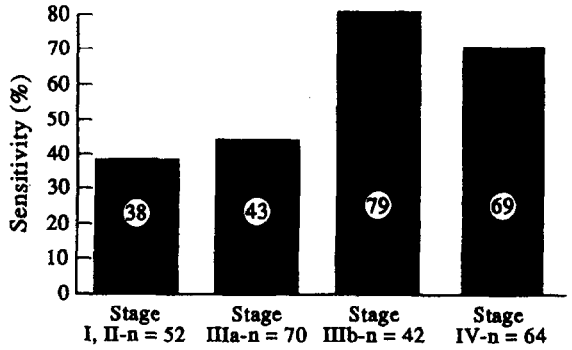

Figure 4. Sensitivity of CYFRA 21–1 in squamous cell lung carcinoma by stage of disease: stage I and II (n = 52), stage IIIa (n = 70), stage IIIb (n = 42) and stage IV (n = 64). The sensitivity is indicated in % in each bar. Note: staging was not available for 49 SCC.

Non-lung cancers

Sensitivity of CYFRA 21-1 was also studied in other cancers. At 3.3 ng/ml, the observed sensitivity was 26% for bladder cancer (n = 23), 26% for head and neck cancers (n = 27), 19% for cervical cancer (n = 47), but only 2% in digestive tract cancers (n = 54) and 6% in breast cancers (n = 16) (Table 3).

Comparison with other markers

The comparison of CYFRA 21-1 sensitivity with that of TPA, SCC and CEA was made in lung cancers at the same 96% specificity using the same benign lung disease population. At this specificity, the cutoffs for CEA, SCC, TPA and CYFRA 21-1 were 7.5 ng/ml, 2.5 ng/ml, 170 U/l and 3.3 ng/ml, respectively. In squamous cell lung carcinoma (n = 277), the sensitivity was 57% for CYFRA 21-1, 46% for TPA, 30% for SCC and 25% for CEA. In adenocarcinoma (n = 172) and undifferentiated large cell carcinoma of the lung (n = 98), the sensitivities were 27 and 34% for CYFRA 21-1; 26 and 34% for TPA; 50 and 40% for CEA, respectively (Table 4).

Table 3. CYFRA 21-1 in other cancers

	Total number	Sensitivity by stage (>3.3 ng/ml) Stage I,II	Stage III,IV	Total sensitivity (%)
Head and neck cancers	27	2/15	5/12	26
Digestive tract cancers				
Oesophagus	21	0/14	1/7	5
Gastric	14	0/7	0/7	0
Colorectal	19	0/7	0/12	0
Gynaecological cancers				
Cervix	47	2/25	7/22	19
Breast	16	0/10	1/6	6
Bladder cancers	23	0/9	6/14	26

3.3 ng/ml refers to the 96% specificity in benign lung diseases.

Table 4. Comparison of sensitivity of different tumour markers in NSCLC histological subtypes

	SCC (%)	Adenocarcinoma (%)	ULCC (%)
CYFRA 21-1 > 3.3 ng/ml	57	27	34
CEA > 7.5 ng/ml	25	50	40
TPA > 170 U/l	46	26	34
SCC > 2.5 ng/ml	30	NA	NA

SCC, squamous cell carcinoma; ULCC, undifferentiated large cell carcinoma; TPA, tissue polypepide antigen; CEA, carcinoembryonic antigen; NA, not available. For each tumour marker the cutoff refers to the 96% specificity in the same benign lung disease group: CYFRA 21-1 (3.3 ng/ml); CEA (7.5 ng/ml); SCC (2.5 ng/ml) and TPA (170 U/l).

More accurate comparison of CYFRA 21-1 and TPA in squamous cell lung cancer was made using ROC curves at 50 to 100% specificity (Figure 5). The area under the curve was 0.87 for CYFRA 21-1 and 0.83 for TPA.

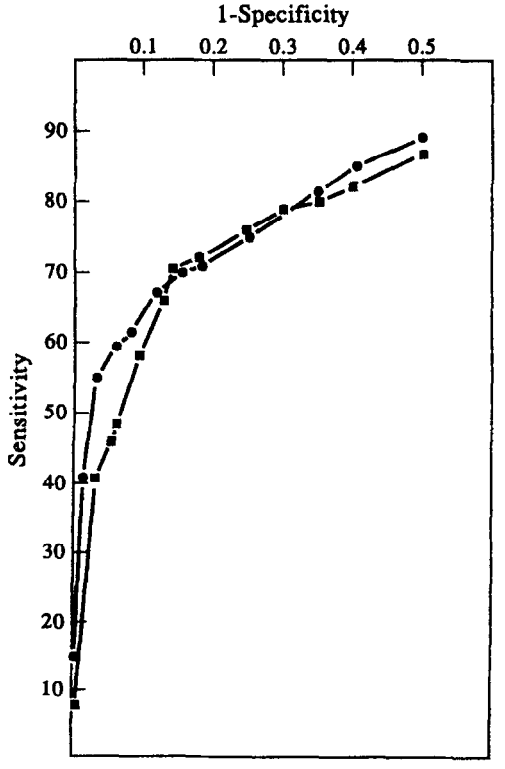

Figure 5. Comparison between TPA and CYFRA 21-1 in squamous cell lung carcinoma by means of ROC curves. Only specificity from 50 to 100% is shown. Specificity is based on the same benign lung disease population (n = 318), and sensitivity is based on the same squamous cell lung carcinoma group (n = 265). ●——● CYFRA 21–1, ■——■ TPA.

DISCUSSION

Ck 19 is a cytoskeletal protein expressed in simple epithelia. Results of immunohistochemical stainings revealed that Ck 19 is more often expressed in NSCLC than in SCLC, and that it is less often expressed in liver diseases than Ck 18 [3]. The results presented here show a very low serum level of CYFRA 21-1 in a large group of healthy blood donors without any influence of smoking habits, sex and age. Interesting results were obtained in the group of benign lung diseases. At diagnosis of primary lung cancer, many patients may also present with non-malignant diseases, such as chronic obstructive bronchopneumopathy, and some will suffer from infectious diseases during treatment. Therefore, a useful lung tumour marker should have a very good specificity. In patients with benign lung diseases, no differences between groups could be found.

CDA curves were used to establish the cutoff value for CYFRA 21-1 in NSCLC. CDA analyses for NSCLC were always compared to CDA analysis of benign lung diseases. By doing so, possible false positives were limited. Furthermore, the CYFRA 21-1 assay was not designed to screen for NSCLC and, therefore, its limited sensitivity at early stages was not a major concern. Our data clearly indicate that CYFRA 21-1 is the tumour marker of first choice for squamous cell lung cancer. In this case, CYFRA 21-1 serum levels are related to the tumour mass. The sensitivities of CYFRA 21-1 for stages IIIa and IIIb are 43 and 79%, respectively. Lower sensitivity of CYFRA 21-1 values in stage IV may be related to metastatic cancers with smaller tumour mass. CYFRA 21-1 assay values were shown to be different between T1T2N0N1 and T3T4N2N3 patients. It was recently suggested that clinical investigations for detecting metastases should only be performed in the latter group [19]. Elevated values of CYFRA 21-1 in asymptomatic T1T2N0N1 patients may indicate the presence of (micro) metastases, and may prompt the clinician to do further clinical investigations.

The study of benign non-lung diseases showed that liver diseases only very weakly influence the serum level of CYFRA 21-1. On the contrary, elevated levels of the marker were detected in cases of renal failure. This may suggest that in healthy persons, Ck 19 is cleared by the kidney.

In non-lung squamous type cancers, we did not find many elevated CYFRA 21-1 serum values for cervical and gastrointestinal cancers. However, in bladder and head and neck cancers, elevated CYFRA 21-1 levels were found in about one third of patients. In squamous cell lung cancer, SCC and CEA showed only weak sensitivities whereas TPA had intermediate sensitivity. In undifferentiated large cell carcinoma and adenocarcinoma, the sensitivity of CYFRA 21-1 was largely comparable to that of TPA; CEA showed a higher sensitivity in these cancers. Therefore, CEA may be a useful lung tumour marker in NSCLC with normal CYFRA 21-1 serum levels.

As the most important use of CYFRA 21-1 will be in therapy monitoring and follow-up of NSCLC patients, further studies are underway to evaluate this potential use of CYFRA 21-1. Independent prognostic value of CYFRA 21-1 was already established in a recent study on 165 prospectively included patients [15]. Based on the high specificity of CYFRA 21-1, we may suggest that this new tumour marker will be useful for NSCLC follow-up. As such, it could overcome the clinical problem of finding false positive elevations during follow-up, a problem recently documented for TPA in bladder cancer [20].

In conclusion, the new tumour marker CYFRA 21-1 is a reliable assay to evaluate the presence of Ck 19 fragments in serum of patients with NSCLC, and particularly in patients with squamous cell lung cancer. The data presented here stimulates us to further efforts to define the clinical usefulness of CYFRA 21-1 for NSCLC therapy monitoring and follow-up.

1. Luna EJ, Hitt AL. Cytoskeleton-plasma membrane interactions. *Science* 1992, **258**, 955–963.
2. Lazarides E. Intermediate filaments as mechanical integrators of cellular space. *Nature* 1980, **283**, 249–256.
3. Moll R, Franke WW, Schiller DL, Geiger B, Krepzer R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 1982, **31**, 11–24.
4. Moll R, Löwe A, Laufer J, Franke WW. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. *Am J Pathol* 1992, **140**, 427–447.
5. Fuchs E, Tyner AL, Giudice G, Marchuk D, Ray Chaudhury A, Rosenberg M. The human keratin genes and their differential expression. In Sawyer RH, ed. *The Molecular and Developmental Biology of Keratins*. New York, Academic Press, 1987, 5–34.
6. Broers JLV, Ramaekers FCS, Rot MK, *et al.* Cytokeratins in different types of human lung cancer as monitored by chain-specific monoclonal antibodies. *Cancer Res* 1988, **48**, 3221–3229.
7. World Health Organization. The World Health Organization histological typing of the lung tumors. *Am J Clin Pathol* 1982, 77, 123–136.
8. Concannon JP, Dalbow MH, Hodgson SE, *et al.* Prognostic value of preoperative carcinoembryonnic antigen (CEA) plasma levels in patients with bronchogenic carcinoma. *Cancer* 1978, **42**, 1477–1483.
9. Carney DN, Ihde DC, Cohen MH, *et al.* Serum neuron-specific enolase: a marker for disease extent and response to therapy of small-cell lung cancer. *Lancet* 1982, **1**, 583–585.
10. Buccheri GF, Violante B, Sartoris AM, Ferrigno D, Curcio A, Vola F. Clinical value of a multiple biomarker assay in patients with bronchogenic carcinoma. *Cancer* 1986, **57**, 2389–2396.
11. Buccheri GF, Ferrigno D, Sartoris AM, Violante B, Vola F, Curcio A. Tumor markers in bronchogenic carcinoma – superiority of tissue polypeptides antigen to carcinoembryonnic antigen and carbohydrate antigenic determinant 19–9. *Cancer* 1987, **60**, 42–50.
12. Buccheri GF, Ferrigno D. Usefulness of tissue polypeptide antigen in staging, monitoring, and prognosis of lung cancer. *Chest* 1988, **93**, 565–570.
13. IUAC. Lung tumours. In Hermanek P, Sobin LH, eds. *International Union Against Chancer – TNM Classification of Malignant Tumours*. Paris, Springer Verlag, 1988, 71–76.
14. Tisi GM, Friedman PJ, Peters RM, *et al.* American Thoracic Society: clinical staging of primary lung cancer. *Am Rev Respir Dis* 1982, **125**, 659–664.
15. Pujol JL, Grenier J, Daver A, Daurés JP, Pujol H, Michel FB. Serum fragment of cytokeratin 19 measured by CYFRA 21-1 immuno-radiometric assay as a marker of lung cancer. *Cancer Res* 1993, **53**, 1–6.
16. Krouwer JS. Cumulative distribution analysis graphs – an alternative to ROC curves. *Clin Chem* 1987, **33**, 2305–2306.
17. Neil Mc BJ, Keeler E, Adelstein SJ. Primer on certain elements of decision making. *N Engl J Med* 1975, **293**, 211–215.
18. Hanley JA, Mc Neil BJ. The meaning and use of the area under receiving operating characteristic (ROC) curve. *Radiology* 1982, **43**, 29–36.
19. Conférence de Consensus de la Société de Pneumologie de Langue Française. Bilan d'extension préthérapeutique du cancer bronchique non à petites cellules. *Bull Cancer* 1992, **79**, 509–517.
20. Van der Gaast A, Kirkels WJ, Blijenberg BG, Splinter TA. Evaluation of tissue polypeptide antigen serum levels for monitoring disease activity during chemotherapy in patients with transitional carcinoma of the urinary tract. *J Cancer Res Clin Oncol* 1992, **118**, 626–628.

APPENDIX

The following are members of the CYFRA 21-1 Multicenter Study Group:

Splinter TWA, van der Gaast A, Blijenberg BG: University Hospital Rotterdam, Dept of Medical Oncology, Dr Molewaterplein, 403015, GD Rotterdam, The Netherlands.
Fombellida Cortazar JC, Genolla Subirats J: Servicio de Medicina Nuclear, Hospital de Cruces, Bilbao, Spain.
Pecchio F, Rapellino M: Ospedale Maggiore S. Giovanni Battista, C. so Bramante, 88/90, 10100 Turin, Italy.
Biersack HJ, Bieker RJ, Schultes BC, Loos U: Universitätsklinik für Nuklearmedizin, Sigmund Freud Str, 25; 5300 Bonn 1, Germany.
De Angelis G*, Cianetti A†: *3e Dept of C. Forlanini Chest Hospital, Via Portuense 332, 00149, †Central Laboratory of S. Camillo Hospital, Circonv. Gianicolense 87, 00152 Rome, Italy.
Scheulen ME: Innere Klinik und Poliklinik (Tumorforschung), Westdeutsches Tumorzentrum, Universitätsklinikum Essen, Huflandstr. 55, D-4300 Essen 1, Germany.
Tuchais C*, Daver A*, Tuchais E†: †Service de Pneumologie CHRU d'Angers, *CRLCC Paul Papin, 2 rue Moll, 49036 Angers, France.
Gaillard G, Gachon F: CRLCC Jean Perrin, 58 rue Montalembert, BP 392, 63011 Clermont-Ferrand Cedex 1, France.
Bonfrer JMG: Department of Clinical Chemistry, The Netherland Cancer Intitute (Antoni van Leeuwenhoek Huis), Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.
Larbre H: Institut Jean Godinot, 1, rue du Général Koenig, BP 171, 51056 Reims Cedex, France.
Allende Ma T, Fernandez Llana B, Ruibal A: Gabinete de Actos Cientificos, Hospital Central de Asturias 33006 Oviedo, Spain.

Pergamon

European Journal of Cancer Vol. 30A, No. 5, pp. 606–610, 1994

0959–8049/94 $7.00+0.00

0959-8049(93)E0038-6

A Pilot Study of Accelerated Cyclophosphamide, Epirubicin and 5-Fluorouracil Plus Granulocyte Colony Stimulating Factor as Adjuvant Therapy in Early Breast Cancer

L. Del Mastro, O. Garrone, M.R. Sertoli, G. Canavese, A. Catturich, M. Guenzi, R. Rosso and M. Venturini

32 consecutive early breast cancer patients were treated to evaluate the feasibility of an accelerated CEF regimen (cyclophosphamide 600 mg/m², epirubicin 60 mg/m² and 5-fluorouracil 600 mg/m²) given intravenously every 2 weeks for six cycles together with granulocyte colony stimulating factor, 5 μg/kg/day subcutaneously from day 4 to day 11. One hundred and eighty two out of 192 planned cycles (95%) were administered. Toxicity was mild: no cases of grade IV non-haematological toxicity and only one episode of grade IV granulocytopenia were observed. Delays or dose reductions of anti-neoplastic drugs occurred in 14 cycles (7.7%). The mean duration of six cycles of treatment was 71 days (planned 70) and 93% of average planned dose intensity was actually administered. The short course CEF therapy is a feasible, well tolerated outpatient chemotherapy regimen, allowing a 46% increase in dose intensity compared with a standard CEF regimen given every 3 weeks. A randomised study comparing this regimen to a standard CEF regimen is now in progress in early breast cancer patients.

Key words: dose intensity, breast cancer, adjuvant chemotherapy, G-CSF
Eur J Cancer, Vol. 30A, No. 5, pp. 606–610, 1994

INTRODUCTION

ONE EXTENSIVELY studied and debated chemotherapy variable over the last few years has been dose intensity. Retrospective analyses indicate that treatment outcome of early breast cancer patients may be affected both by the total dose of chemotherapy actually administered [1] and by the dose intensity of the utilised regimen [2]: the higher the dose or the dose intensity, the better the outcome. However, until now, no randomised study has proven that high dose intensity chemotherapy regimens are better than standard.

Actually, the most important limiting factor hindering the administration of higher than standard dose intensity is myelotoxicity. Recently, some haemopoietic colony stimulating factors (CSFs) have proven able to reduce the haematological toxicity of some standard dose regimens [3, 4]. Moreover, several phase I/II studies utilising granulocyte (G-CSF) or granulocyte–