TUMOR MARKER CYFRA-21-1 IN SERUM AND PLEURAL EFFUSIONS OF PATIENTS WITH LUNG CANCER

Davor Jurman [1], Ljerka Lukinac [2], Dunja Nöthig-Hus [2], Dražena Krilić [2], Dragutin Cvrtila [2], Josip Lukač [2] and Zvonko Kusić [2]

[1]Department of Pulmonology, University Department of Medicine, [2]University Department of Oncology and Nuclear Medicine, Sestre milosrdnice University Hospital, Zagreb, Croatia

SUMMARY – Tumor marker CYFRA-21-1 may be used as an additional parameter in the diagnosis, prognosis and follow-up of patients with non-small cell lung carcinoma (NSCLC), especially those with squamous cell carcinoma. From 1994 till 2001, the concentration of CYFRA-21-1 was determined in serum and/or pleural effusions of 166 patients with NSCLC, and in serum of 28 control subjects. Serum CYFRA-21-1 median in the control, benign and malignant group was 0.8 ng/mL, 2.6 ng/mL and 6.3 ng/mL, respectively ($p>0.05$). In pleural effusions, CYFRA-21-1 median differed significantly between the benign (8.2 ng/mL) and malignant (146 ng/mL) group ($p<0.0000$). The overall CYFRA-21-1 sensitivity and specificity were 62% and 76% in serum, and 73% for both parameters in pleural effusions. Our results confirmed the CYFRA-21-1 to better discriminate benign from malignant pleural effusions than benign from malignant sera.

Key words: *Lung neoplasms, blood; Tumor markers – biological, blood; Lung neoplasms, pathology; Pleural effusion – malignant, diagnosis*

Introduction

Among malignant diseases, lung cancer is most widespread. In the USA, it kills 28% of the people with malignancy (32% male and 25 % female)[1]. In Croatia, in the year 1998, an incidence of 56/100000 and mortality of 54/100000 were recorded. The major risk factors for lung cancer are cigarette smoking, genetic predisposition, influence of arsenic, chrome, asbestos, x-ray radiation, air pollution, etc[2]. According to histological typing, there are four main groups of lung cancer: squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and small cell carcinoma. The first three categories are combined into the group of non-small-cell lung carcinoma (NSCLC), which include about 80% of all lung carcinomas[3].

Correspondence to: *Davor Jurman, M.D.* , Department of Pulmonology, University Department of Medicine, Sestre milosrdnice University Hospital, Vinogradska c. 29, HR-10000 Zagreb, Croatia

Received November 19, 2002, accepted December 30, 2002

In the diagnosis of lung cancer, different methods have been used, e.g., cytologic, histologic, endoscopic, radiologic, scintigraphic, etc. Determination of the tumor markers of carcinoembryonic antigen (CEA), neuron specific enolase (NSE) or cytokeratin 19 fragment (CYFRA-21-1) may serve as a useful, additional parameter not only for the diagnosis but even more for the prognosis and monitoring of the patient's disease[4].

Tumor marker CYFRA-21-1 is a fragment of cytokeratin 19, which is part of cytoskeleton in epithelial cells. If the tumor is of epithelial origin, CYFRA-21-1 can be released into the bloodstream. CYFRA-21-1 has been shown to have high sensitivity in patients with NSCLC, especially those with squamous cell lung carcinoma[5,6].

Differential diagnosis of pleural effusion is a common clinical problem. It is not easy to confirm the pleural effusion malignancy only on the basis of cytologic analysis. To find a minimally invasive tool for differentiating between pleural effusion of malignant and benign origin, some authors assessed several tumor markers individually or in

combination[7]. The aim of this study was to assess whether an increased Cyfra 21-1 level in serum or in pleural effusion may be an independent diagnostic factor of existing malignancy.

Material and Methods

In this retrospective study carried out from 1994 till 2001, CYFRA-21-1 was determined in serum, pleural effusion, and both serum and pleural effusion of patients with benign non-lung disease and patients with lung cancer, and also in sera of healthy subjects (controls). Patients with inflammation and those with liver, kidney and heart disease were included in the benign group. Patients with squamous cell lung carcinoma prevailed in the malignancy group.

Pleural effusion malignancy was confirmed by cytologic examination.

Blood and pleural effusion samples were centrifuged to discard the cell pellet and were subsequently aliquoted and frozen (-20 °C) until analysis.

Controls and patients

Control group included 28 healthy blood donors, 8 male and 20 female (aged 41-85 years).

Of 166 patients (aged 34-87 years) included in the study, there were 101 male and 65 female patients, 47 with benign disease and 119 with malignant disease (squamous cell lung carcinoma in 66, adenocarcinoma in 30, undifferentiated carcinoma in 18, microcellular cancer in three, and lymphoma in two patients). Except for nine patients, all had only one sample of serum or pleural effusion analyzed for CYFRA-21-1; in eight of these nine patients CYFRA-21-1 level was determined twice, and in one patient CYFRA-21-1 was measured on three occasions.

The levels of CYFRA-21-1 were determined in total of 131 serum samples and 63 pleural effusions. In addition to Cyfra-21-1, other tumor markers, i.e. CEA, carbohydrate antigen 15-3 (CA 15-3) and carbohydrate antigen 125 (CA 125) were in parallel determined in 33 pleural effusions.

Method

ELSA CYFRA-21-1 (Schering Cis bio international, Gifsur-Yvette, France; cutoff <3.3 ng/mL) is a solid phase "sandwich" immunoradiometric assay[8] for detection of soluble cytokeratin 19 fragments in the effusion. Two monoclonal antibodies (BM 19-21 and KS 19-1 obtained after immunization of mice with MCF-7 cells) prepared against sterically remote sites on the CYFRA-21-1 molecule, the first being coated on the ELISA solid phase and ther other radiolabelled with iodine ^{125}I, were used as a tracer.

The concentrations of CEA, CA15-3 and CA 125 were measured by the immunoradiometric assay (CEA IRMA, cutoff <5.0 ng/mL; CA 15-3 IRMA, cutoff <35.3 U/mL; CA125 IRMA, cutoff <26.0 U/mL, all from DPC, Los Angeles, CA, USA).

Statistics

Medians of CYFRA-21-1 levels were compared by the χ^2-test. A p value <0.05 was considered significant. The receiver-operating characteristic (ROC) curve was used to determine the sensitivity and specificity of the groups compared.

Results

Unfortunately, it was not possible to collect both serum and pleural effusion samples from each patient included in the study. Therefore, CYFRA-21-1 was assayed on an unbalanced number of serum and pleural effusion sam-

Table 1. Concentrations of CYFRA-21-1 in serum

Group	n	Median (ng/mL)	Range (ng/mL)	<3.3* n	Specificity (%)	>3.3 n	Sensitivity (%)
Control	28	0.8	0.3- 3.2	0	100		
Benign	21	2.6	0.3- 16.7	16	76		
Malignant	82	6.3	0.1-138			51	62
a) adenocarcinoma	7	17.4	2.6-138			5	71
b) squamous ca	60	6.3	0.1-104			39	65
c) other types ca	15	4.6	0.9-108			8	53

* cutoff in serum <3.3 ng/mL

Table 2. Concentrations of CYFRA-21-1 in pleural effusions

Group	n	Median (ng/mL)	Range (ng/mL)	<3.3* n	Specificity (%)	>50** n	Sensitivity (%)
Control							
Benign	26	10.7	0.1- 78	19	73		
Malignant	37	146	8.4-3280			27	73
a) adenocarcinoma	23	154	8.4-3280			19	83
b) squamous ca	6	233	12.2-1664			4	67
c) other types ca	8	52	31.4-2400			4	50

* cutoff in serum <3.3 ng/mL, ** cutoff in pleural effusion <50 ng/mL

ples of patients with benign and malignant disease, which may have caused a problem in the interpretation of the statistical analysis applied.

For 28 healthy blood donors (controls), serum CYFRA-21-1 median was 0.8 ng/mL, range 0.18-2.5 ng/mL. At a cutoff serum value of 3.3 ng/mL, the specificity was 100%. In 21 patients with benign disease, serum CYFRA-21-1 median was 2.6 ng/mL and the corresponding specificity was 76%. A total of 82 patients with malignant lung disease had serum median of 6.3 ng/mL (Table1). Median of the dominant tumor type, squamous cell carcinoma (n=60), was 6.3 ng/mL, with the corresponding sensitivity of 65%. Median values were not statistically significantly different ($p>0.05$).

The pleural effusion CYFRA-21-1 median for 26 patients with benign disease was 10.7 ng/mL, and for 37 patients with malignancy 146 ng/mL (Table 2), yielding a statistically significant difference ($p<0.0000$).

In adenocarcinoma that prevailed in the patient group (n=23), the median was 154 ng/mL. At a cutoff CYFRA-21-1 value of 50 ng/mL for pleural effusion[9-11], the corresponding sensitivity was 83%.

The highest serum CYFRA-21-1 median of 17.4 ng/mL was detected in adenocarcinoma, and highest pleural effusion median of 233 ng/mL in the group of squamous cell carcinoma.

The median CYFRA-21-1 values in serum and pleural effusion were compared between the study groups. A statistically significant difference ($p<0.0000$) was recorded only between the benign group and adenocarcinoma, and between adenocarcinoma and squamous cell lung carcinoma in serum and pleural effusion.

Comparison of CYFRA-21-1 concentrations between the benign group and malignant pleural effusions was presented by means of ROC curve (Fig. 1). The following parameters were obtained: area under the curve (AUC) of 0.943; 95% confidence interval (CI: 0.854-0.985); sensitivity of 89.2%; and specificity of 88.5%.

In 12 frozen pleural effusion samples from adenocarcinoma patients with breast cancer metastases, apart from CYFRA-21-1, tumor markers CA15-3 and CA125 were also determined.

The median values of 76 ng/mL, 52.3 U/mL and 498 U/mL for CYFRA-21-1, CA15-3 and CA125 all exceeded the respective serum cutoff values.

In a total of 21 pleural effusions, CEA was determined in parallel to CYFRA-21-1. The median values of CYFRA-21-1 and CEA were 23.2 ng/mL and 3.6 ng/mL ($p<0.0000$) in the benign group (n=12), and 185 ng/mL and 97.3 ng/mL in the malignant group (n=8).

Changes in CYFRA-21-1 concentrations (5 in serum and 4 in pleural effusions) were monitored in only eight patients on two visits and in one patient on three visits.

Discussion

Cytokeratins belong to a group of intermediate filament proteins, which are a major component of the cell skeleton. There are around 20 different cytokeratins with a molecular mass ranging from 40 to 70 kilodaltons (kD). Low molecular mass is found in simple epithelium and heavy molecular mass in epidermis[12].

Several cytokeratins including cytokeratin 19 are connected with malignancy. In patients with lung cancer it may be released into the serum as fragments measured by the CYFRA-21-1 assay. The measurement of this tumor marker is a noninvasive method for improving the diagnosis of malignant lung cancer[4,13,14]. However, CYFRA-21-1 was not efficient in either screening or detection of early stage NSCLC, but has been used as an independent marker of squamous cell carcinoma of the lung[5]. Nowadays, some research is carried out to evaluate CYFRA-21-1 level in malignant pleural effusion.

Our initial results on CYFRA-21-1[15] in patients with squamous cell lung carcinoma showed 80% of patients with distant metastases (stage IIIB and stage IV) to have an increased serum CYFRA-21-1 concentration, supporting other authors[16] who found highest CYFRA-21-1 values in metastatic lung cancer and highest sensitivity in stage IIIB and IV squamous cell carcinoma. Clinical evaluation of CYFRA-21-1 in pleural effusion has been described by Satoh *et al.*[17]. They found higher CYFRA-21-1 median in malignant (84.5 ng/mL) than in benign (13.9 ng/mL) diseases. Our results are similar (146 ng/mL *vs.* 10.7 ng/mL).

Of the four tumor markers (CYFRA-21-1, CEA, SCC and NSE) relevant for the detection of lung cancer, Muraki *et al.*[14] found highest sensitivity for CYFRA-21-1. For adenocarcinoma, Lai *et al.*[13] recorded serum CYFRA-21-1 sensitivity of only 39%. In the present study, we found a sensitivity of 83% in pleural effusion and of 71% in serum. In spite of the small number of serum samples of adenocarcinoma, we obtained a significant difference between the benign disease group and adenocarcinoma in both serum and pleural effusion ($p<0.0000$). In contrast to other authors[4], our data indicated that CYFRA-21-1 might be a relevant tumor marker for adenocarcinoma. Toumbis *et al.*[9] have confirmed the CYFRA-21-1 level in pleural fluid to be 1 to 385 times higher than in serum of the benign and malignant lung diseases examined. We found the Cyfra-21-1 level in malignant pleural fluid to be up to 24-fold (range 8.4-3280 ng/mL) maximal concentration in malignant serum (range 0.1-138 ng/mL). According to ROC analysis (Fig. 1), CYFRA-21-1 levels from the benign and malignant pleural fluids were well discriminated. The role of tumor markers in differentiating malignant from benign pleural effusion is not yet clear, as stated by Romero *et al.*[10]. In pleural fluid, they found low CYFRA-21-1 sensitivity of only 32% (specificity 82%). Therefore, they conclude that CYFRA-21-1 is useless in pleural fluid and that only a combination of CEA, CA 15-3 and Cyfra-21-1 in serum may obviate its determination in pleural fluid. Other authors[18] confirmed that out of four tumor markers measured (AFP, ferritin, CEA and CYFRA-21-1) only CEA and CYFRA-21-1 had diagnostic value in differentiating benign from malignant pleural effusion. When pleural effusion cytology is negative, CYFRA-21-1 and CEA may be useful assays in the diagnosis of malignant pleural effusion[19]. However, Alata *et al.*[20] did not prove the diagnostic value of tumor markers CEA, CA15-3, CA19-9, CYFRA-21-1, NSE and TSA in pleural effusions.

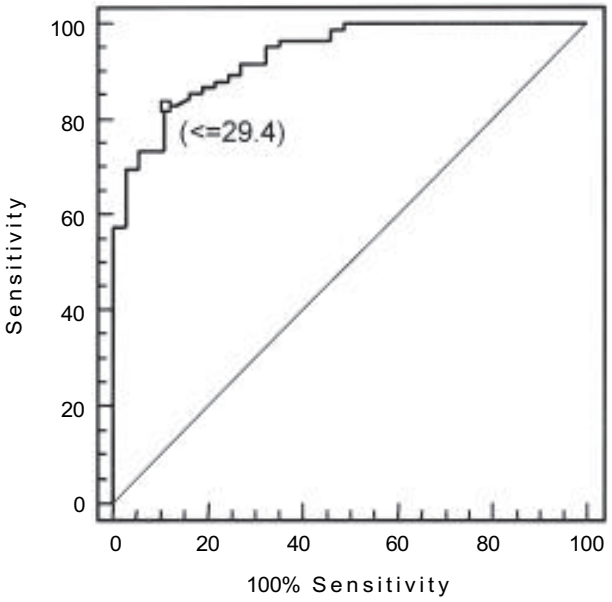

Fig. 1 Receiver operating characteristic curve for CYFRA 21-1 in benign and malignant pleural effusions (area under the curve AUC = 0.943,95% CI = 0.854 – 0.985; sensitivity = 89.2% specificity = 88.5%)

The results obtained by tumor marker combinations (CYFRA-21-1 and CEA in parallel in benign and malignant groups, and CYFRA-21-1, CA15-3 and CA 125 in parallel in patients with breast carcinoma) determined in pleural fluids of 33 samples confirmed the concentrations of all four tumor markers to be increased in patients with malignancy. The median values in the benign and malignant groups were highly significant ($p<0.0000$), those of CYFRA-21-1, CA15-3 and CA 125 all greatly exceeded their serum cutoff values.

It is well known that the most important role of CYFRA-21-1 is in therapy monitoring and follow-up of patients with NSCLC[4,21]. Unfortunately, until now we have had an opportunity to monitor CYFRA-21-1 level twice in only eight patients and three times in one patient with lung cancer. In three patients with benign disease at the first visit the pleural effusion CYFRA-21-1 concentration was increased but it declined on the second visit. Two out of five patients with squamous cell lung carcinoma had CYFRA-21-1 values below the normal level in both serum samples. In one patient serum level increased significantly, and in the other two CYFRA-21-1 levels remained highly increased. In one patient with adenocarcinoma both CYFRA-21-1 values in pleural effusions were highly increased. According to such a low rate of CYFRA-21-1 determination, and lacking data on therapy and clinical examinations of these patients, we are not yet able to evaluate CYFRA-21-1 results during the follow-up period.

Conclusion

The results obtained confirm that CYFRA-21-1 is a useful parameter in distinguishing benign from malignant pleural effusions and malignant sera from malignant pleural effusions. The highest median concentration of CYFRA-21-1 was detected in serum of patients with adenocarcinoma and in pleural effusions of patients with squamous cell lung carcinoma. Their CYFRA-21-1 median values were significantly different ($p<0.0000$).

In the near future, Cyfra-21-1 concentration should be evaluated in monitoring therapeutic efficacy in patients with lung cancer. Another application of CYFRA-21-1 determination might be in patients with pulmonary peripheral solid lesion which is unaccessible by transthoracic needle. If CYFRA-21-1 is increased, explorative thoracotomy should be considered.

References

1. LANDIS SH, MURRAY T , BOLDENS S , *et al*. Cancer statistics 1999. CA Cancer J Clin 1999; 49:8-31.
2. GRBAC I, BAŠIĆ -GRABAC M, OSTOJIĆ J. Lung carcinoma. Medicus 2001;10:179-80.
3. MOUNTAIN CF . A system for the clinical staging of lung cancer . Am J Roentgenol Radium Ther Nucl Med 1974;120:130.
4. RASTEL D, RAMAIOLI A, CORNILLIE F , THIRION B on behalf on the Cyfra 21-1 multicentre study group. Cyfra 21-1, a sensitive and specific new tumor mark er for squamous cell lung cancer . Report of the first European multicentre evaluation. Eur J Cancer 1994;30:601-6.
4. PUJOL JL, GRENIER J, DA URES JP, DAVER A, PUJOL H, MICHEL FB. Serum fragment of cytok eratin subunit 19 measured by Cyfra 21-1 immunoradiometric assay as a mark er of lung cancer . Cancer R es 1993;53:61-6.
6. PUJOL JL, BOHER JM, GRENIER J, QU ANTIN X. Cyfra 21-1, neuron specific enolase and prognosis of non-small cell lung cancer: prospective study in 621 patients. L ung Cancer 2001;31:221-31.
7. VILLENA V, LOPEZ-ENCUENTRA A, ECHA VE-SUSTAETA J, MARTIN-ESCRIBANO P , ORTUNO -DE-SOLO B, ESTENOZ -ALFARO J. Diagnostic value of CA 72-4, carcinoembryonic antigen, CA15-3, and CA 19-9 assay in pleural fluid. A study of 207 patients. Cancer 1996;78:736-40.
8. ROUX N, ROUX F , FERRAND V, KLEISBAUER JP. Validation methodologique et clinique d'une trousse de dosage immunoradiometrique d'un nouveau marqueur du cancer du poumon: le Cyfra 21-1. Immunoanal Biol Spec 1994;9:30-9.
9. TOUMBIS M, RASID AKIS A, PASSALIDOU E, KALOMENIDIS J, ALCHANA TIS M, ORPHANIDOU D, JORD ANOGLOU J. Evaluation of CYFRA -21-1 in malignant and benign pleural effusions. Anticancer R es 1996;16:201-4.
10. ROMERO S, FERNANDEZ C, ARRIERO JM, ESP ASA A, CANDELA A, MAR TIN C, SANCHEZ -PAYA J. CEA, CA 15-3 and CYFRA -21-1 in serum and pleural fluid of patients with pleural effusions. Eur R espir J 1996;9:17-23.
11. LAI RS, CHEN CC, LEE PC, LU JY. Evaluation of cytok eratin 19 (CYFRA -21-1) as a tumor mark er in malignant pleural effusion. Jpn J Clin Oncol 1999;29:421-4.
12. FUCHS E, TYNER AL, GIUDICE G, MARCHUK D, RAY CHA UDHUR Y A, ROSENBERG M. The human k eratin genes and their different e xpression. In: SAWYER RH, ed. The molecular and developmental biology of k eratins. New Y ork: Academic P ress, 1987:5-34.
13. LAI RS, HSU HK, LU JY , GER LP, LAI NS. CYFRA -21-1 enzyme-linked immunosorbent assay . Evaluation as a tumor mark er in non-small cell lung cancer . Chest 1996;109:995-1000.
14. MURAKI M, TOHD A Y, IWANAGA T, UEJIMA H, NAGASAK A Y, NAKAJIMA S. Assessment of serum CYFRA -21-1 in lung cancer . Cancer 1996;77:1274-7.
15. LUKINAC L j, JURMAN D, NÖTHIG -HUS D, NOVOSEL S, KUSIĆ Z. Tumor mark er CYFRA -21-1 in lung cancer patients 1996;21:P-4. Book of Abstracts, Second International Congress of the Croatian Society of Nuclear Medicine, Zagreb, 1996.
16. RAPELLINO M, NIKLINSKI J, PECCHIO F , FURMAN M, BAL -DI S, CHY CZEWSKI L, RUFFINI E, CHY CZEWSKA E. CYFRA -21-1 as a tumor mark er for bronchogenic carcinoma. Eur R espir J 1995;8:407-10.
17. SATOH H, SUMI M, YAGYU H, ISHIK AWA H, SUY AMA T, NAITOH T, SAITOH T, HASEGA WA S. Clinical evaluation of CYFRA -21-1 in malignant pleural fluids. Oncology 1995;52:211-4.
18. MALEŠIČ I, POHAR J. Tumorski biljezi u pleuralnim izljevima. Biochemia Medica 1996;6:64 (S6/6).
19. SALAMA G, MIEDOUGE M, ROUZA UD P, MAUDUYT MA, PUJAZON MC, VINCENT C, CARLES P , SERRE G. Evaluation of pleural CYFRA -21-1 and carcinoembryonic antigen in the diagnosis of malignant pleural effusions. Br J Cancer 1998;77:472-6.
20. ALATA F, ALATA Ö, METINT A M, COLAK Ö, HARMANCI E, DEMIR S . Diagnostic value of CEA, CA 15-3, CA19-9, CYFRA -21-1, NSE and TSA assay in pleural effusions. L ung Cancer 2001;31:9-16.
21. TAKEI Y, MINA TO K, TAKISE A, TSUCHIY A S, SAITO R, MORI M. CYFRA -21-1 is an useful mark er of survival and therapeutic effect in lung cancer . Cancer Detect P rev 1998;*22* (Suppl 1):465 (Abstract).

Sažetak

TUMORSKI BILJEG CYFRA -21-1 U SERUMU I PLEURALNOM IZLJEVU B OLESNIKA S KARCINOMOM PL UĆA

D. Jurman, Lj. Iukinac, D. Nöthig-Hus, D. Krilić, D. Cvrtila, J. Iukač i Z. Kusić

Za dijagnosticiranje, prognozu i praćenje bolesnika s karcinomom pluća, osobito skvamoznog tipa, poznato je određivanje koncentracije tumorskog biljega CYFRA -21-1. U razdoblju od 1994. do 2001. godine izmjerena je razina CYFRA -21-1 u serumu ili/i pleuralnom izljevu 166 bolesnika, od kojih je 119 imalo karcinom pluća, a 47 benignu bolest. Medijani koncentracije CYFRA -21-1 u serumu kontrolne, benigne i maligne sk upine iznosili su 0,8 ng/mL, 2,6 ng/mL i 6,3 ng/mL i nisu se značajno razlikovali ($p>0,05$). Nasuprot tome, u pleuralnom izljevu medijani CYFRA -21-1 benigne i maligne sk upine od 8,2 ng/mL i 146 ng/mL bili su značajno različiti ($p<0,0000$). Osjetljivost i specifičnost CYFRA -21-1 u serumu bila je 62% odnosno 76%, a u pleuralnom izljevu su oba parametra iznosila 73%. Naši dosadašnji rezultati potvrđuju da je CYFRA -21-1 koristan dodatni parametar u razlikovanju benignih od malignih pleuralnih izljeva, ali ne i benignih od malignih seruma.

Ključne riječi: *Plućne neoplazme, krv; Tumorski biljezi – biološki, krv; Plućne neoplazme, patologija; Pleuralni izljev – maligni, dijagnostik*