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

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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 carcinomas³.

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 disease⁴. 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 carcinoma⁵,⁶. 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. 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.

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<th>Table 1. Concentrations of CYFRA-21-1 in serum</th>
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<td><strong>Group</strong></td>
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* cutoff in serum <3.3 ng/mL.
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 (Table 1). 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, 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.

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. 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. Nowadays, some research is carried out to evaluate CYFRA-21-1 level in malignant pleural effusion.
Our initial results on CYFRA-21-1 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 authors15 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 Sato 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 authors4, 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 authors18 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 effusion19. 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.

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 NSCLC21. 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.

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%)
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

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


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/pleuralnom izljevu 166 bolesnika, od kojih je 119 imalo karcinom pluća, a 47 benigne bolesti. 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 obe parametre 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