# ONCOLOGY

# Diagnostic Value of Tumor Markers Cyfra 21-1 and Neuron-Specific Enolase in Analysis of Pleural Fluid

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The diagnostic value of tumor markers Cyfra 21-1 and neuron-specific enolase in blood serum and pleural fluid in differential diagnosis of pleural exudation in cancer patients and patients with nontumor pleurisy was evaluated. The most pronounced changes were characteristic of Cyfra 21-1. In patients with pleurisy caused by malignant tumors the degree and incidence of increased Cyfra 21-1 concentrations in the serum and pleural fluid were higher than in patients with pleural exudation of nontumor origin. These data attest to high diagnostic sensitivity and specificity of Cyfra 21-1. Complex measurements of the marker in the serum and pleural fluid will improve diagnosis of pleural exudation of tumorous etiology.

**Key Words:** *tumor markers; Cyfra 21-1; neuron-specific enolase; pleural exudation*

Tumorous pleurisy is diagnosed on the basis of cytological analysis of pleural exudation, which sometimes is little informative. Tumor markers (carcinoembryonic antigen, $\beta_2$-microglobulin, CA 15-3, *etc.*) were used to improve the accuracy of pleural exudation diagnosis [5,6,8]. The possibility of measuring Cyfra 21-1 (Cyfra) and neuron-specific enolase (NSE) in the serum and pleural exudation for differential diagnosis of pleurisy was demonstrated [4,5,7,9,11].

Cyfra is a new tumor marker, belonging to cytokeratins. Its clinical use became possible after development of enzyme immunoassay for detection of cytokeratin 19 fragments (soluble cytokeratin) in the serum and other biological fluids. Cytokeratin 19 is a cytoskeleton component of epithelial cell; expression of this marker was also demonstrated for mesothelial cells. Hyperexpression of Cyfra is characteristic of malignant tumors of different location, primarily squamous cell carcinoma of the lung, oral cavity, and urinary bladder [3,10]. Measurement of Cyfra in the serum of patients with lung cancer is now used for the diagnosis of squamous-cell variant of this disease [1,3,10].

NSE is an isozyme of glycolytic enzyme enolase, highly prevalent in the central and peripheral nervous system cells. Due to pronounced expression in neuroendocrine tumors, NSE is used as a tumor marker for the diagnosis and monitoring of pulmonary small-cell carcinoma [1,2].

There are virtually no objective data in Russian literature on the possibility of using tumor markers, along with other methods of investigation, for differential diagnosis of pleural exudation. We measured Cyfra and NSE in pleural fluid and serum for differential diagnosis of pleural exudation caused by malignant tumors and nontumor diseases.

## MATERIALS AND METHODS

Patients with pleural exudation of tumor ($n$=32) and nontumor ($n$=37) etiology and age-matched healthy men and women ($n$=30) were examined. Patients with pleural exudation caused by histologically confirmed

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malignant tumors (aged 31-90 years) presented with lung cancer ($n$=11), breast cancer ($n$=4), ovarian cancer ($n$=4), pancreatic cancer ($n$=1), lymphosarcoma ($n$=1), melanoma ($n$=1), and lung and pleura metastases from initially unknown focus ($n$=10). Patients with pleural exudation of nontumor origin were aged 31-85 years (mean age 69.9 years); 22 patients presented with stages II-III circulatory insufficiency, 8 with pneumonia, 4 with tuberculosis, and 3 with posttraumatic pleurisy.

Cyfra and NSE tumor markers were detected in the serum and pleural fluid of patients by two- and single-step streptavidin enzyme immunoassay (Enzymun-Test Cyfra 21-1 and Enzymun-Test NSE) using ES-300 system (Boehringer Mannheim). Normal values of serum Cyfra and NSE were taken for 3.3 and 12.5 ng/ml, respectively, in accordance with the manufacturer's recommendations and our data.

The significance of differences between means was evaluated using Student's $t$ test, the differences were considered significant at $p<0.05$; analysis of correlations was carried out by Spearman nonparametrical $R$ test using Statistica for Windows 5.5 software.

## RESULTS

Comparative analysis of the concentrations of tumor markers in the serum and pleural fluid showed that the most marked changes were characteristic of Cyfra.

Serum concentrations of Cyfra increased significantly in cancer patients compared to healthy subjects and patients with nontumor pleurisy (Table 1). Serum concentration of NSE in cancer patients, patients with nontumor diseases, and normal subjects was virtually the same (12.5 ng/ml) (Table 1).

Changes of tumor markers in the serum and pleural fluid were different. The concentrations of Cyfra in pleural exudation in patients with pleurisy of tumor and nontumor origins 11.4- and 5.8-fold surpassed those in the sera, while the concentration of NSE in pleural fluid of patients of both groups was lower than in the serum (Table 1). These regularities reflect different mechanisms of appearance of NSE and Cyfra in biological fluids and confirm specificity of NSE as a marker of small-cell lung carcinoma. Further analysis included only measurements of Cyfra as a potential marker of pleurisy.

Generally, the mean level of Cyfra in the pleural fluid of cancer patients was 7.8 times higher than in patients with pleurisy of nontumor origin (Table 1). In patients with lung cancer the concentration of Cyfra (283.7±102.6 ng/ml) was 3-fold higher than in patients with cancer of other locations (92.2±23.2 ng/ml, $p<0.05$). However, the mean concentrations of Cyfra in the sera of these patients were virtually the same (13.9 and 14.5 ng/ml), which more than 4-fold surpassed the normal. This fact confirms expression of Cyfra in malignant tumors of different histogenesis and substantiated the diagnostic value of this marker in all cancer patients.

The concentrations of Cyfra in the serum and pleural fluid of cancer patients varied within a wide range (Table 1). On the other hand, in patients with nontumor pleurisy these fluctuations were less pronounced; the highest serum concentrations were observed in pneumonia (16.1 ng/ml) and the highest concentrations in pleural fluid — in circulatory insufficiency (95.6 ng/ml).

For analysis of the diagnostic value of Cyfra in differential diagnosis of pleural exudation caused by malignant tumors, threshold values of the marker in

**TABLE 1.** Concentrations (ng/ml) of Cyfra and NSE in Sera and Pleural Fluid of Patients with Cancer and Nontumor Diseases

| Object of analysis | | Control ($n$=30) | Patients | |
|---|---|---|---|---|
| | | | with malignant tumors ($n$=32) | with nontumor diseases ($n$=37) |
| Pleural fluid | | | | |
| Cyfra | $M\pm m$ | — | 162.4±42.8$^{+}$ | 20.9±3.9 |
| | range | | 3.0-903.0 | 0.45-95.60 |
| NSE | $M\pm m$ | — | 7.8±1.5$^{+}$ | 3.4±0.7 |
| | range | — | 0.57-37.4 | 0.6-15.6 |
| Serum | | | | |
| Cyfra | $M\pm m$ | 1.5±0.2 | 14.3±3.4* | 3.6±0.5* |
| | range | 0.7-3.3 | 1.1-75.0 | 0.6-16.1 |
| NSE | $M\pm m$ | 9.3±0.2 | 16.1±1.8* | 12.4±0.9* |
| | range | 5.9-12.0 | 2.6-48.6 | 6.3-27.1 |

**Note.** $p<0.05$: *compared to the control, $^{+}$compared to patients with nontumor diseases.

**TABLE 2.** Distribution of Patients with Malignant Tumors and Nontumor Diseases Depending on Cyfra Concentrations in Pleural Fluid

| Cyfra concentration, ng/ml | Patients with malignant tumors (*n*=32) | | Patients with nontumor diseases (*n*=37) | |
|---|---|---|---|---|
| | abs. | % | abs. | % |
| Below 10 | 4 | 12.5 | 19 | 51.4 |
| 10.1–20.0 | 5 | 15.6 | 9 | 24.3 |
| 20.1-40.0 | 4 | 12.5 | 3 | 8.1 |
| 40.1–75.0 | 5 | 15.6 | 5 | 13.5 |
| Above 75.0 | 14 | 43.8 | 1 | 2.7 |

the serum and pleural fluid were calculated on the basis of values in patients with nontumor pleurisy. In accordance with the standard requirements to statistical analysis, the threshold levels of Cyfra were calculated with consideration for the mean value and two standard deviations ($M \pm 2SD$), which corresponded to 95% confidence interval. The threshold level of Cyfra in pleural fluid was 75.0 ng/ml, in the serum — 9.6 ng/ml.

The concentration of Cyfra in pleural exudation of patients with nontumor diseases surpassed 75 ng/ml only in circulatory insufficiency and in only 1 of 22 patients. In none patients with pneumonia, tuberculosis, and pleural injury the concentration of Cyfra in the pleural exudation surpassed 75 ng/ml. On the whole, the increase in Cyfra concentration was observed in only 2.7% patients with pleurisy of unknown etiology, which indicates high specificity (97.3%) of this marker concetration as a threshold value (Table 2). Serum Cyfra concentration surpassed 9.6 ng/ml in only 2 (5.4%) patients with pneumonia.

Increased concentrations of Cyfra in pleural fluid and serum were found in 43.8 and 31.2% patients with pleurisy caused by malignant tumors, respectively (Table 2). Complex analysis of the marker in both biological fluids increased the diagnostic sensitivity of Cyfra to 62.1%, the specificity remained high (92%).

Hence, our study demonstrated high sensitivity and specificity of Cyfra as a tumor marker; measurements of this marker in pleural fluid and serum will improve the accuracy of differential diagnosis of pleural exudation of tumor and nontumor origin.

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