Clinical Usefulness of Serum Cytokeratin 19 Fragment as a Tumor Marker for Lung Cancer

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Serum soluble cytokeratin 19 fragment (CYFRA) levels were measured in 251 patients with lung cancer and 139 patients with benign lung diseases to determine the clinical usefulness of CYFRA level determination in the diagnosis and monitoring of lung cancer. Serum levels of CYFRA were measured by a 2-step sandwich ELISA method. When the cut-off value was defined as 3.5 ng/ml, which was associated with a specificity of 95% for benign lung diseases, CYFRA had a high sensitivity (53%) in all patients with lung cancer. Both the serum level of CYFRA and its sensitivity increased significantly with the increase in clinical stage. A comparison of areas under receiver operating characteristic curves showed that CYFRA had the most power of discrimination in the diagnosis of lung cancer among markers including carcinoembryonic antigen, squamous cell carcinoma antigen, carbohydrate antigen 19-9, and neuron-specific enolase. A good correlation was found between serial changes in serum CYFRA levels during therapy and clinical responses for 18 patients who underwent chemotherapy and/or radiotherapy. Our findings suggest that CYFRA may be a marker of choice for screening and monitoring of lung cancer, particularly squamous cell carcinoma.

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Key words: carcinoembryonic antigen, squamous cell carcinoma antigen, carbohydrate antigen 19-9, neuron-specific enolase, non-small cell lung cancer, squamous cell carcinoma

**Introduction**

Lung cancer is the leading cause of cancer deaths in the world. Non-small cell lung cancer (NSCLC) accounts for the majority (75–80%) of all lung cancers. At the time of diagnosis, approximately 30% of patients with NSCLC are eligible for surgical resection, but the remaining 70% have little chance of cure. There is therefore a great need for tumor markers for early diagnosis and follow-up of NSCLC. A number of tumor markers, including carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCC), carbohydrate antigen (CA) 19-9, and neuron-specific enolase (NSE), are used for the diagnosis and monitoring of lung cancer. However, these markers have been found to be insufficient for the definitive diagnosis of lung cancer due to their low sensitivities for early-stage disease.

In this study, we examined the usefulness of soluble cytokeratin 19 fragment (CYFRA) as a tumor marker for lung cancer.

**Materials and Methods**

**Patients**

This retrospective study employed serum samples obtained from 251 patients with lung cancer and 139 patients with benign lung diseases (Tables 1 and 2). They comprised a portion of patients who had been admitted to National Shikoku Cancer Center Hospital between January 1991 and December 1994. The patients with lung cancer included 184 males and 67 females (median age, 68 years at diagnosis) and those with benign lung diseases included 74 males and 65 females (63 years). All the patients with lung cancer underwent a series of examinations for staging, and were classified using the tumor-node-metastasis system (1); stage I disease was found in 62 patients, II in 12, IIIA in 41, IIIB in 67, and IV in 69. Histological classification of the tumor was based on the World Health Organization (WHO) criteria (2): there were 110 squamous cell carcinomas, 98 adenocarcinomas, 10 large cell carcinomas, and 33 small cell carcinomas. Seventy-eight patients underwent...
Table 1. Patients with Lung Cancer

<table>
<thead>
<tr>
<th>Histological type</th>
<th>I</th>
<th>II</th>
<th>IIIA</th>
<th>IIIB</th>
<th>IV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>33</td>
<td>6</td>
<td>24</td>
<td>21</td>
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<tr>
<td>Adenocarcinoma</td>
<td>25</td>
<td>2</td>
<td>22</td>
<td>36</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Patients with Benign Lung Disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia/lung abscess</td>
<td>60</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>31</td>
</tr>
<tr>
<td>Benign lung tumor</td>
<td>17</td>
</tr>
<tr>
<td>Chronic obstructive lung disease</td>
<td>8</td>
</tr>
<tr>
<td>Other lung diseases*</td>
<td>23</td>
</tr>
</tbody>
</table>

*Other lung diseases included 6 patients with sarcoidosis, 5 with bronchial asthma, 4 with interstitial pneumonia, 3 with pulmonary mycosis, 3 with middle lobe syndrome, and 2 with pneumothorax.

Measurement of serum levels of CYFRA and other tumor markers

Serum samples were obtained from patients both at the time of diagnosis and at evaluation of tumor response, and stored at -70°C until use. The serum levels of CYFRA were determined using a 2-step sandwich ELISA kit (Enzymun-Test CYFRA 21-1, Boehringer-Mannheim, Germany). Serum levels of CEA, SCC, CA 19-9, and NSE were determined as part of a routine medical check at admission. The levels of the former 3 markers were measured with EIA kits (EIA-Test CEA II “BMY”, Boehringer-Mannheim; IMX SCC DAINAPACK, Dainabot, Japan; Enzymun-Test CA 19-9, Boehringer-Mannheim), and that of NSE was measured with an RIA kit (NSE EIKEN, EIKEN KAGAKU, Japan). The cut-off value of CYFRA was set at 3.5 ng/ml in accordance with the 95% specificity approach, as recommended by the Hamburger Group for the Standardization of Tumor Markers (4). The cut-off values for the other markers were set at 6.7 ng/ml for CEA, 1.9 ng/ml for SCC, 43.9 U/ml for CA 19-9, and 10.7 ng/ml for NSE, based on the same specificity value as for CYFRA.

Surgical resection and 160 patients underwent chemotherapy and/or radiotherapy, but the remaining 13 were ineligible to receive any anticancer therapy, mainly due to poor clinical condition. Performance status (PS) was classified using the Eastern Cooperative Oncology Group scale. Tumor responses to chemotherapy and/or radiotherapy were classified in accordance with the WHO criteria (3).

Results

Serum levels of CYFRA and histological types of lung cancer

The serum level of CYFRA was 10.92±1.39 ng/ml in patients with lung cancer, and significantly higher than that (1.92±0.10 ng/ml) in patients with benign lung diseases (p<0.001). By histological type of lung cancer, the levels of CYFRA were 14.6±2.46 ng/ml for squamous cell carcinoma, 8.92±1.98 ng/ml for adenocarcinoma, 3.62±0.72 ng/ml for large cell carcinoma, and 6.77±2.65 ng/ml for small cell carcinoma (Fig. 1). The levels of CYFRA were significantly higher in patients with each histological type of lung cancer than in those with benign lung diseases (p<0.001). In addition, the levels of CYFRA were significantly higher in patients with squamous cell carcinoma and NSCLC (11.55±1.55 ng/ml) than in those with small cell carcinoma (p<0.001). CYFRA levels were not correlated with sex, age, or smoking index for patients with benign lung diseases (data not shown).

Serum levels of CYFRA by clinical stage of lung cancer

Serum levels of CYFRA in patients with NSCLC were 4.58±0.83 ng/ml for stage I, 5.98±1.85 ng/ml for stage II, 8.20±1.76 ng/ml for stage IIIA, 11.6±1.72 ng/ml for stage IIIB, and 21.9±5.19 ng/ml for stage IV disease (Fig. 2a). The level of CYFRA increased significantly with the increase in clinical stage (p<0.001). Similarly, levels of CYFRA in patients with squamous cell carcinoma were 6.30±1.42 ng/ml for stage I, 9.22±2.83 ng/ml for stage II, 9.37±2.29 ng/ml for stage IIIA, 13.4±2.51 ng/ml for stage IIIB, and 36.7±10.98 ng/ml for stage IV disease (Fig. 2b). A significant difference between the levels of CYFRA and clinical stage was observed (p=0.012).

Sensitivities of CYFRA and other tumor markers for lung cancer

The sensitivities of CYFRA and other markers for detection of various types of lung cancer are shown in Fig. 3. The

Statistical analysis

Values are means±SEM. Except for the chi-square test and receiver operating characteristic (ROC) analysis, nonparametric methods were used; correlation coefficients were determined by Spearman's rank-correlation test; the significance of differences between two independent groups was determined by the Mann-Whitney U test; and the significance of differences among more than two groups was determined by Kruskal-Wallis one-way analysis. The probability of survival from the initiation of therapy was estimated using the method of Kaplan and Meier, and the test of difference in survival distribution was based on the generalized Wilcoxon test. p values of less than 0.05 were considered statistically significant.

The ROC curves were constructed and the areas under the curve for each marker were calculated using the CLABROC program (5). For comparisons of the accuracies of two different markers, the areas under their ROC curves were compared using the univariate z-score test.
The sensitivities of CYFRA were 53.0% for all lung cancer, 57.3% for NSCLC, 66.4% for squamous cell carcinoma, 46.9% for adenocarcinoma, 50.0% for large cell carcinoma, and 24.2% for small cell carcinoma. The sensitivity of CYFRA for NSCLC was significantly higher than those of CEA (39.5%), SCC (27.1%), CA 19-9 (18.0%), and NSE (19.0%) (p<0.01). Similarly, the sensitivity of CYFRA for squamous cell carcinoma was significantly higher than those of CEA (25.2%), SCC (43.7%), CA 19-9 (13.4%), and NSE (18.2%) (p<0.01). In addition, the sensitivity of CEA for adenocarcinoma (58.0%) and that of NSE for small cell carcinoma (58.6%) were particularly high.

Figure 4 shows sensitivities of CYFRA and other markers by clinical stage of NSCLC (Fig. 4a) and squamous cell carcinoma.
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(Fig. 4b). The sensitivities of CYFRA were higher than those of other markers for every stage, and the differences were statistically significant for most comparisons (data not shown). In addition, the sensitivities of CYFRA for stage I and II diseases were 38.7% and 41.7% for all lung cancer, 38.3% and 45.5% for NSCLC, and 54.5% and 83.3% for squamous cell carcinoma.

To further investigate the usefulness of CYFRA in early diagnosis of lung cancer, we examined the sensitivity of CYFRA at the cut-off value of 2.6 ng/ml, which corresponded to a specificity value of 85% for benign lung diseases. At this setting, the cut-off values for the other markers were 5.4 ng/ml for CEA, 1.2 ng/ml for SCC, 24.5 U/ml for CA 19-9, and 8.1 ng/ml for NSE. The sensitivities of CYFRA were increased for the groups of all lung cancers (70.3%), NSCLC (72.3%), and squamous cell carcinoma (82.7%) when compared with those at the cut-off value of 3.5 ng/ml. Of note, the sensitivity of CYFRA was particularly high for stage I (72.7%) and stage II (100%) squamous cell carcinoma.

ROC curves for CYFRA and other tumor markers

Figure 5 shows ROC curves for CYFRA and other markers for all lung cancer patients (Fig. 5a), those with NSCLC (Fig. 5b), and those with squamous cell carcinoma (Fig. 5c). The ROC curves for CYFRA were clearly superior to those for the other markers for all three groups. For the group of all lung cancer patients, the areas under the ROC curves were 0.8508±0.0190 for CYFRA, 0.7540±0.0254 for CEA, 0.6547±0.0308 for SCC, 0.5914±0.0353 for CA19-9, and 0.7132±0.0306 for NSE. For patients with NSCLC, they were 0.8610±0.0189 for CYFRA, 0.7705±0.0257 for CEA, 0.6805±0.0311 for SCC, 0.6105±0.0357 for CA19-9 and 0.6830±0.0330 for NSE, while for patients with squamous cell carcinoma, they were 0.9168±0.0177 for CYFRA, 0.7252±0.0330 for CEA, 0.7895±0.0323 for SCC, 0.6210±0.0424 for CA19-9 and 0.6726±0.0388 for NSE. In addition, significant differences were found between CYFRA and other markers in the three groups when the areas under their ROC curves were compared (p<0.01).

Correlations between serum levels of CYFRA and other tumor markers

Serum levels of CYFRA were not correlated with those of CEA (r=0.36), SCC (r=0.47), CA19-9 (r=0.25), or NSE (r=0.29) (Fig. 6).

Changes in serum levels of CYFRA during therapy

Serum samples were obtained both at the time of diagnosis and at evaluation of the tumor response (maximal tumor response of therapy or disease progression) from 18 patients. All of these patients underwent chemotherapy and/or radiotherapy: one obtained complete remission, 10 obtained a partial response, and the remaining 7 had stable disease or disease progression. Changes in serum CYFRA levels during therapy are shown by tumor response in Fig. 7. For the group of 11 patients whose tumor responded, serum levels of CYFRA decreased significantly during therapy (p<0.01), while levels of CYFRA were unchanged for the group of 7 patients in whom the tumor did not respond.

Survival of state IV-NSCLC patients according to the serum level of CYFRA

Of the 58 patients with stage IV-NSCLC, the 43 who underwent chemotherapy were evaluated for tumor response and survival. Fourteen had a normal serum level of CYFRA (≤3.5 ng/ml), while 29 had an elevated level (>3.5 ng/ml). Their characteristics are listed by level of CYFRA in detail in Table 3. There were no differences in clinical background or tumor response between these two groups. The median survival time
Discussion

Cytokeratins are major components of the intermediate filament network, and together with actin, myosin, and microtubules compose the cellular cytoskeleton in epithelial cells (6). Cytokeratin 19 is a 40 kDa acidic cytokeratin, and is highly expressed in simple epithelium of the lung (6). Interestingly, the expression of cytokeratin 19 is enhanced during malignant transformation, and cytokeratin 19 is released from cancer cells following tumor lysis and necrosis (7), suggesting that cytokeratin 19 might be useful as a tumor marker for lung cancer.

Due to the high sensitivity and low tissue-specificity of CYFRA for lung cancer, it has been considered primarily a “pan-marker” for lung cancer (8). However, in our series, CYFRA had a significantly higher sensitivity for NSCLC (57.3%) and squamous cell carcinoma (66.4%) than for other histological types of lung cancer. Similar findings have been reported by other authors (8–14). These findings suggest that CYFRA is a useful marker for NSCLC, and an especially useful
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Figure 6. Correlations between serum levels of CYFRA and other tumor markers. Serum levels of CYFRA were not correlated with those of CEA (r=0.36), SCC (r=0.47), CA 19-9 (r=0.25), or NSE (r=0.29).

In this study, we used a cut-off value for CYFRA which corresponded to 95% specificity for benign lung diseases, as recommended by the Hamburger Group for the Standardization of Tumor Markers (4). The cut-off value for CYFRA was defined as 3.5 ng/ml in this setting, and its sensitivity was 53% for lung cancer. This sensitivity is similar or slightly superior to the values of 40% (9), 46% (10), 47% (8), and 48% (11) obtained in previous studies using the same specificity. In addition, the sensitivity of CYFRA for lung cancer increased to 70.3% when the cut-off value corresponding to 85% specificity was used. At this value of specificity, Sugama et al (12) and Takada et al (10) also reported high sensitivities of 58% and 65%, respectively.

The serum level of CYFRA increased significantly with the increase in clinical stage, suggesting that the CYFRA level reflects the tumor burden. Notably, the sensitivity of CYFRA was high in early-stage squamous cell carcinoma (54.5% for stage I disease and 83.3% for stage II disease). It was higher when the cut-off value corresponding to 85% specificity was used (72.7% for stage I disease and 100% for stage II disease). Lai et al (11) and Pujol et al (13) reported that CYFRA was not
Figure 7. Changes in serum levels of CYFRA during therapy according to tumor response. Serum levels of CYFRA significantly decreased during therapy for the group of 11 patients in whom the tumor exhibited a response to treatment (Mann-Whitney, \( p<0.01 \)), but were unchanged for the group of 7 patients in whom the tumor did not respond. CR: complete remission, NC: no change, PD: progressive disease, PR: partial response.

Figure 8. Survival curves for 43 patients with stage IV non-small cell lung cancer as determined by the serum level of CYFRA. Median survival times were 22 months for the 14 patients with a normal level of CYFRA (<3.5 ng/ml) and 10 months for the 29 patients with an elevated level of CYFRA (>3.5 ng/ml) (generalized Wilcoxon, \( p=0.46 \)).

Table 3. Characteristics of Patients with Stage IV Non-Small Cell Lung Cancer

<table>
<thead>
<tr>
<th>CYFRA ≤3.5 ng/ml</th>
<th>CYFRA &gt;3.5 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>14</td>
</tr>
<tr>
<td>Male/female</td>
<td>7/7</td>
</tr>
<tr>
<td>Performance status: 0–1/2–3</td>
<td>12/2</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>63 (45–76)</td>
</tr>
<tr>
<td>Weight loss*: &lt;5%/≥5%</td>
<td>12/2</td>
</tr>
<tr>
<td>Histological type</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>5</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>9</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>0</td>
</tr>
<tr>
<td>Response: PR/NC/PD</td>
<td>7/7/0</td>
</tr>
</tbody>
</table>

*Percent decrease of body weight during the last 6 months before diagnosis. NC: no change, PD: progressive disease, PR: partial response.

helpful in screening or early diagnosis of lung cancer, although our data have encouraged us to use CYFRA for these purposes. The sensitivity of CYFRA was clearly influenced by the proportions of various benign lung diseases included in the benign disease group. Sugama et al (12) reported that the serum level of CYFRA is elevated in patients with pneumonia, tuberculosis, and interstitial pneumonia, but it is low in those with chronic obstructive lung disease, sarcoidosis, an bronchial asthma. The group of patients with benign lung disease in the present study included large proportions of patients with pneumonia (43%) and tuberculosis (22%), and a small proportion of those with interstitial pneumonia (3%). The possibility that the inclusion of a small proportion of patients with interstitial pneumonia affected the cut-off value of CYFRA in our study cannot be excluded. However, the groups with benign lung disease in the reports by Lai et al (11) and Pujol et al (13) included no patients with interstitial pneumonia. Therefore, the high sensitivity of CYFRA for the detection of early-stage squamous cell carcinoma found in our study cannot be explained by the proportion of patients included with benign lung disease, and is inconsistent with the reports by Lai et al (11) and Pujol et al (13).

CYFRA thus exhibited a high degree of sensitivity for lung cancer, and particularly for squamous cell carcinoma, but its sensitivities for adenocarcinoma (46.9%) and small cell carcinoma (24.2%) were less than those of CEA (58.0%) and NSE (58.6%). We examined the diagnostic accuracy of tumor markers with ROC curves. A comparison of the curves clearly demonstrated the superiority of CYFRA to CEA, SCC, CA19-9, and NSE for the three groups of lung cancers studied.

A good correlation was found between serial changes in CYFRA during therapy and the clinical responses for the 11
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patients who obtained a clinical response to treatment. A similar result was reported by Takada et al (10). For other markers such as CEA, serial measurement is useful for monitoring the response to therapy and for predicting recurrence of tumor (15–17). Therefore, a large-scale study will be needed to clarify the role of CYFRA in such usage.

Pujol et al (13) and Moro et al (18) found lower survival for patients with lung cancer who had an elevated level of CYFRA than for those without elevated CYFRA. The significance of the difference in survival was confirmed by multivariate analysis, and in the latter study CYFRA level was the factor with the strongest effect on survival in NSCLC. Similarly, in our series, stage IV-NSCLC patients with a normal level of CYFRA had longer survival than did those with an elevated CYFRA, although the difference between these groups was not significant. These findings suggest that the CYFRA level is useful for the staging of lung cancer and for the determination of its prognosis.

In conclusion, our findings suggest that CYFRA may be the marker of choice for screening and monitoring of lung cancer, particularly squamous cell carcinoma.

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References