Analysis of Tumor Markers in the Cytological Fluid Obtained from Computed Tomography-Guided Needle Aspiration Biopsy for the Diagnosis of Non-small Cell Lung Cancer

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Purpose: The aim of this study was to prospectively assess whether analysis of the tumor markers cytokeratin 19 fragments (CYFRA 21-1), carcinoembryonic antigen (CEA), and squamous cell carcinoma (SCC) antigen in cytological fluid can improve the performance of computed tomography (CT)-guided needle aspiration biopsy (NAB) in the diagnosis of non-small cell lung cancer (NSCLC).

Methods: A total of 100 patients (men:women = 41:59, mean age: 63 years) with suspected malignant pulmonary lesions were prospectively enrolled for CT-guided NAB procedures. Levels of CYFRA 21-1, CEA, and SCC in the cytological fluid were measured by immunoradiometric assays. The cutoff value for tumor markers was selected on the basis of best accuracy through receiver operating characteristic curves. The sensitivity and areas under the curve (AUC) of NAB alone were compared with those of NAB combined with cytological tumor markers (CYFRA 21-1, CEA, and SCC).

Results: Among 100 patients, 71 (71%) had NSCLC and 29 (29%) had benign lesions. The sensitivity, specificity, and accuracy for diagnosing NSCLC were 85.7%, 100%, and 89%, respectively, for NAB alone. The sensitivity increased significantly for NAB combined with a tumor marker compared with NAB alone (100% for CYFRA 21-1, 92.9% for CEA, and 94.2% for SCC; \( p < 0.001 \), \( p = 0.025 \), and \( p = 0.014 \), respectively). The AUC of NAB with CYFRA 21-1 was significantly larger than the AUC of NAB alone (\( p = 0.001 \)).

Conclusion: Evaluation of tumor markers CYFRA 21-1, CEA, and SCC in the cytological fluid can improve the diagnostic performance of CT-guided NAB for NSCLC. Of these markers, CYFRA 21-1 is the most useful cytological tumor marker.

Key Words: CYFRA 21-1, CEA, SCC-Ag, Tumor marker, Cytological fluid, CT-guided needle aspiration biopsy (NAB).

Lung cancer is one of the most prevalent and life-threatening neoplasms in most parts of the world.1,2 Lung cancer survival and individual therapeutic approaches largely depend on the histology and stage of the disease at diagnosis,3 therefore early and accurate diagnosis of lung cancer is important. In clinical practice, diagnostic tools commonly used for lung cancer are computed tomography (CT) scans, bronchoscopy, and sputum analysis, which all have limitations in the early diagnosis of lung cancer. Therefore, biopsy with histopathological examination is usually used to confirm the diagnosis of lung cancer.2

CT-guided needle aspiration biopsy (NAB) of the lung is a relatively safe and accurate method for diagnosing lung lesions, even small lesions.4 The reported accuracy is relatively high, ranging from 64 to 97%, and major complications are rare; however, transthoracic needle biopsy of lung lesions has a false-negative rate of up to 20% in the diagnosis of malignancy,5,6,7 and results revealing nonspecific benign tissue or insufficient tissue for diagnosis are often not reliable in excluding malignancy. Patients with these types of biopsy results should have resampling of tissue with biopsy or surgical resection, or close clinical and imaging follow-up.6

Tumor markers in the serum have been extensively studied in lung cancer. Measurement of tumor marker concentrations is a much simpler and safer method than biopsy or surgery for the diagnosis of lung cancer.7 Several tumor markers including cytokeratin 19 fragments (CYFRA 21-1), carcinoembryonic antigen (CEA), and squamous cell carcinoma (SCC) antigen have been investigated for their diagnostic and prognostic value in non-small cell lung cancer (NSCLC); however, there are no valuable tumor markers.
for lung cancer screening. Tumor markers in lung cancer are useful tools in the follow-up of patients of cancer and are used mainly for monitoring the efficacy of therapy and in the early detection of recurrence.\(^7\)\(^9\) One of the main drawbacks of serum tumor markers is the fact that high concentrations are usually only found when the disease is at an advanced stage.\(^10\)\(^11\) Therefore, it is very difficult to clinically detect a lung tumor at an early stage with serum marker assays.\(^10\)\(^12\)

Among the many possible types of samples for tumor marker analysis, cytological fluid obtained from NAB has the potential to be an effective source, as it is obtained directly from tumor tissue, thus many candidate biomarkers will be present in high concentrations. The aim of this study was to prospectively assess whether analysis of tumor markers CYFRA 21-1, CEA, and SCC in the cytological fluid can improve the diagnostic performance of CT-guided NAB for NSCLC.

METHODS

Patient Selection

The study protocol was approved by the institutional review boards, and written informed consent was obtained from all patients.

From May 1, 2009, to October 31, 2009, 115 patients who had a pulmonary nodule or mass suspicious for lung malignancy on CT were prospectively enrolled in this study. The inclusion criteria were age more than 20 years, lesion size more than 8 mm, and solid lesions (ground glass opacity component of <50%). The exclusion criteria were a high index of suspicion for benign disease (e.g., tuberculosis)\(^ (n = 6)\), histologically confirmed small cell lung cancer or lymphoma\(^ (n = 4)\), and refusal to provide written informed consent\(^ (n = 5)\).

The prebiopsy evaluation included reviews of CT scans, laboratory studies, and medical records. All patients underwent CT-guided NAB procedures, and all malignancies had histologically and/or cytologically confirmed NSCLC. The final study population comprised 59 men and 41 women, aged 34 to 82 years (mean age, 63 years). Data collection was systematized, and a standardized registration form was prepared. For each patient, the following information was recorded: age, sex, history, biopsy site, size of nodule, NAB results, pathology results, and laboratory data (cytological fluid tumor markers CYFRA 21-1, CEA, and SCC).

CT-Guided NAB Technique

The biopsy procedures were performed by three experienced chest radiologists who had 3, 5, and 9 years of experience performing thoracic biopsies. CT-guided biopsy interventions were performed using a 16-MDCT scanner (Somatom Sensation 16; Siemens Medical Solutions, Erlangen, Germany) equipped with CARE Vision software (Siemens Medical Solutions). The exposure parameters were 120 kV, 30 mAs, and a slice thickness of 6 mm. All procedures were performed with the patients in a prone, supine, or lateral decubitus position, depending on the location of the lesion. The puncture area was cleaned with antiseptic solution followed by administration of local anesthetic by subcutaneous injection of 1% lidocaine (Xylocaine, AstraZeneca, Wilmington, DE). In all cases, more than two aspiration specimens were obtained to get enough specimen using 20- to 22-gauge Chiba needles. Cytological fluid was also aspirated during the procedure without additional needle punctures. Both aspiration specimens and cytological fluid were obtained with one needle puncture. A part of specimen was placed in 99% ethyl alcohol for cytological examination, the rest of specimen and fluid was prepared in tube for evaluation of cytological tumor marker and immediately transferred for diagnosis.

Tumor Marker Analysis

Cytological fluid was collected from each patient before any therapy. Cytological fluid supernatants were obtained by centrifugation at 2000g for 10 minutes and stored at -40°C before assaying for tumor markers using commercial immunoassay kits. The technicians performing the assays were blinded to the final diagnosis associated with the samples. CYFRA 21-1 levels were measured using an electrochemiluminescent immunoassay (CYFRA 21-1; Roche Diagnostics, Germany), CEA levels were measured using a chemiluminescent immunoassay (Centaur CEA; Bayer HealthCare, Tarrytown, NY), and SCC-Ag levels were measured using an immunoradiometric assay (SCC-RIAEAD; SRL Inc., Japan). Tumor markers in each of the cytological fluid samples were assayed twice, and the mean values were used for analysis.

Statistical Analysis

Cytological results were evaluated and divided into the following diagnostic categories: “malignant,” “suspicious for malignancy,” “negative for malignancy,” and “nondiagnostic” (e.g., cell paucity or samples with a few atypical cells). A designation of “malignancy” or “suspicious for malignancy” was considered a positive result. A designation of “negative for malignancy” was considered a negative result. Nondiagnostic designations\(^ (n = 9)\) were considered neither positive nor negative, and the results were excluded from calculations of sensitivity, specificity, and accuracy. A positive NAB result was considered a true-positive result if there was surgical confirmation and a false-positive result if no evidence of malignancy was found during surgical resection (in the absence of preoperative chemotherapy). Negative results were considered true negative if regression was found on subsequent CT or if no tumor was identified during examination of the surgical specimen. Surgical confirmation of malignancy was considered a false-negative finding.

Receiver operating characteristics (ROC) curves were constructed using the values of tumor markers in the cytological fluid, and a cutoff value was determined for differentiation between malignant and benign lesions. The cutoff level for each marker was selected based on the best diagnostic accuracy; if several cutoff points had the same accuracy, the value with the best specificity was chosen. Differences between the two groups (malignant and benign groups) were evaluated using the \(\chi^2\) test or Fisher’s exact test. The sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) of NAB alone and NAB combined with cytological tumor markers (CYFRA 21-1, CEA, and SCC) were calculated. When analyzing the
diagnostic yield of a combination of tumor markers, a case was considered positive if at least one tumor marker was positive and negative if all tumor markers were negative. We also classified malignant cases according to tumor stage based on the 7th Edition of lung cancer stage classification suggested by the International Association for the Study of Lung Cancer. The sensitivity, specificity, accuracy, PPV and NPV of NAB alone and NAB combined with cytological tumor markers (CYFRA 21-1, CEA, and SCC) were also calculated according to tumor stage. To compare the performance of NAB alone and NAB combined with tumor markers, ROC curves were constructed, and the area under the curve (AUC) was compared. Comparisons were made using the McNemar test for statistical significance of sensitivity, accuracy, and AUC. Statistical analyses were performed with SAS software (version 9.1.3 for Windows; SAS Institute, Cary, NC), and p values less than 0.05 were considered statistically significant.

RESULTS

Among 100 patients, 71 (71%) had NSCLC, and 29 (29%) had benign lesions. Table 1 summarizes patient characteristics in the benign group, malignant group, and the total population. There was no significant difference in any of the characteristics, including age, sex, history of hyper tension or diabetes, tuberculosis status, and the size and location of lesions between the two groups (p > 0.05) (Table 1).

Of the 71 malignant lesions, 41 lesions were confirmed by surgery such as pneumonectomy or lobectomy and 30 were confirmed by biopsy. Of the 29 benign lesions, six were confirmed by video-assisted thoracic surgery or wedge resection, 10 were confirmed as benign clinically, and 13 were eventually diagnosed as benign based on subsequent CT scans that showed lesion regression. One case of malignancy (adenocarcinoma [AC]) and eight cases of benign lesions were nondiagnostic in cytological results. The histological types of NSCLC, according to the WHO classification, were as follows: 54 patients with AC, 11 patients with SCC, one patient with large cell carcinoma, and five patients with NSCLC not otherwise specified.

The cytological fluid concentrations of CYFRA 21-1, CEA, and SCC were 90.5 ± 137 ng/ml (range, 1.67–500), 16.9 ± 40.3 ng/ml (range, 0.1–233.79), and 21.3 ± 45.2 ng/ml (range, 0.07–197.55) for malignant lesions and 10.9 ± 11.08 ng/ml (range, 1.24–47.7), 0.8 ± 1.26 ng/ml (range, 0.1–5.84), and 4.5 ± 14.5 ng/ml (range, 0.07–74.7) for benign lesions, respectively. Levels of CYFRA 21-1, CEA, and SCC in the cytological fluid were significantly higher in the malignant group than in the benign group (p < 0.05).

Using the ROC curves, the best cutoff values for the tumor markers in the cytological fluid were 15.7 ng/ml for CYFRA 21-1, 0.6 ng/ml for CEA, and 0.86 ng/ml for SCC (Figure 1). Table 2 describes the sensitivity, specificity, PPV, accuracy, specificity, and PPV of NAB alone and NAB combined with cytological tumor markers in 91 patients. The sensitivity, specificity, accuracy, PPV, and NPV of NAB alone and NAB combined with cytological tumor markers in 91 patients. The sensitivity, specificity, accuracy, PPV, and NPV of NAB alone were 85.7%, 100%, 89%, 100%, and 67.7%, respectively. The sensitivity increased significantly for NAB combined with a tumor marker compared with NAB alone (100% for CYFRA 21-1, 92.9% for CEA, and 94.2% for SCC: p = 0.001, p = 0.025, and p = 0.014, respectively) (Table 2). The diagnostic accuracy improved significantly for NAB combined with CYFRA 21-1 compared with NAB alone (97.8% versus 89%, p = 0.0209). Nevertheless, the accuracy of NAB combined with CEA or SCC and the accuracy of NAB alone were not significantly different (p = 0.738 and p = 0.527, respectively).

When combinations of tumor markers were evaluated, the sensitivity increased significantly for any combination of tumor markers compared with NAB alone (p < 0.001). Nevertheless, accuracy was not significantly improved with any combination of tumor markers compared with NAB alone (p > 0.05) (Table 2). The sensitivity of NAB with CYFRA 21-1 was not significantly different from that of NAB with any combination of tumor markers (p > 0.05) (Table 2).

Among the 71 malignant lesions, there were 38 cases of stage 1, four cases of stage 2, 11 cases of stage 3, and 18 cases of stage 4. In the results of subgroup analysis according to tumor stage, the sensitivity, specificity, accuracy, PPV, and NPV of NAB alone were 78.9%, 100%, 86.4%, 100%, and 72.4% for stage 1, 100%, 100%, 100%, 100%, and 100% for stage 2, 100%, 100%, 100%, 100%, and 100% for stage 3 and 88.8%, 100%, 94.8%, 100%, and 91.4% for stage 4, respectively. In stage 1, the sensitivity increased significantly for NAB combined with a tumor marker compared with NAB alone (100% for CYFRA 21-1, 89.5% for CEA, and 89.5% for SCC: p = 0.002, p = 0.040, and p = 0.040, respectively). Nevertheless, the accuracy was no significantly different between NAB alone and NAB combined with tumor markers (96.6% for CYFRA 21-1, 86.4% for CEA, and 86.4% for SCC: p > 0.05). In stages 2, 3, and 4, the sensitivity and accuracy were not significantly different between NAB alone and NAB combined with a tumor markers (p > 0.05). For diagnosis of NSCLC, the AUC of NAB with CYFRA 21-1 was significantly larger than the AUC of NAB alone (0.993 versus 0.928, p = 0.001). Nevertheless, the AUC of NAB with CEA or SCC was not significantly larger than the AUC.

<table>
<thead>
<tr>
<th>TABLE 1. Patient Characteristics (N = 100)</th>
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<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td>Mean age (±SD)</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Tuberculosis</td>
</tr>
<tr>
<td>Mean lesion size (mm)</td>
</tr>
<tr>
<td>Location</td>
</tr>
<tr>
<td>Upper/middle lobe</td>
</tr>
<tr>
<td>Lower lobe</td>
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</table>

* Fisher exact test.
of NAB alone (0.950 versus 0.928, \( p = 0.062 \) and 0.957 versus 0.928, \( p = 0.057 \)). The AUC of NAB with any combination of tumor markers was significantly larger than the AUC of NAB alone (\( p < 0.05 \)). Nevertheless, the AUC of NAB with CYFRA 21-1 and the AUC of NAB with any combination of tumor markers including CYFRA 21-1 were not significantly different (\( p > 0.05 \)) (Table 3).

**DISCUSSION**

Our study demonstrates that additional evaluation of cytological tumor markers (CYFRA 21-1, CEA, and SCC) can improve sensitivity in the diagnosis of NSCLC in patients undergoing CT-guided NAB. Among the three tumor markers, NAB combined with CYFRA 21-1 demonstrated the highest sensitivity (100%) and accuracy (97.8%) for diagnosis of NSCLC.

The prognosis of NSCLC is poor; only 5% of patients at clinical stage IIIb and almost none at clinical stage IV survive for 5 years.\(^6\) Early detection of stage I A lung cancer can increase the 5-year survival to 80%, compared with 15% for overall NSCLC.\(^2,^3\) Early detection of malignant small nodules can lead to early treatment that can potentially be curative.\(^15\) In clinical practice, CT-guided biopsy of lung nodules is often performed to obtain a definitive diagnosis\(^6\) and is known to be a useful procedure for diagnosing pulmonary nodules that are highly likely to be malignant, especially in patients who are not candidates for surgery. Some authors have reported that CT-guided biopsy has become an increasingly accurate and sensitive technique for diagnosing pulmonary masses and propose that it should be used for initial diagnosis.\(^16\)

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**TABLE 2.** Comparison of the Diagnostic Results of NAB Alone and NAB Combined with Cytological Fluid Tumor Markers in 91 Patients

<table>
<thead>
<tr>
<th>Diagnostic Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAB alone</td>
<td>85.7</td>
<td>100.0</td>
<td>89.0</td>
<td>100.0</td>
<td>67.7</td>
</tr>
<tr>
<td>NAB + tumor marker</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYFRA (15.7)</td>
<td>100.0</td>
<td>90.4</td>
<td>97.8</td>
<td>97.2</td>
<td>100.0</td>
</tr>
<tr>
<td>CEA (0.6)</td>
<td>92.9</td>
<td>81.0</td>
<td>90.1</td>
<td>94.2</td>
<td>77.2</td>
</tr>
<tr>
<td>SCC (0.86)</td>
<td>94.2</td>
<td>81.0</td>
<td>91.2</td>
<td>94.5</td>
<td>81.0</td>
</tr>
<tr>
<td>CYFRA + CEA</td>
<td>100.0</td>
<td>76.1</td>
<td>94.5</td>
<td>93.3</td>
<td>100.0</td>
</tr>
<tr>
<td>CYFRA + SCC</td>
<td>100.0</td>
<td>80.9</td>
<td>95.6</td>
<td>94.6</td>
<td>100.0</td>
</tr>
<tr>
<td>CEA + SCC</td>
<td>97.1</td>
<td>71.4</td>
<td>91.2</td>
<td>91.9</td>
<td>88.2</td>
</tr>
<tr>
<td>CYFRA + CEA + SCC</td>
<td>100.0</td>
<td>71.4</td>
<td>93.4</td>
<td>92.1</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Numbers in parentheses are cutoff levels of tumor markers in ng/ml.

Nine cases of nondiagnostic results were excluded from calculations of sensitivity, specificity, accuracy, PPV, and NPV.

PPV, positive predictive value; NPV, negative predictive value; CEA, carcinoembryonic antigen; SCC, squamous cell carcinoma; CYFRA, cytokeratin 19 fragments; NAB, needle aspiration biopsy.

**TABLE 3.** Comparison of the Diagnostic Performance of NAB Alone and NAB with Cytological Fluid Tumor Markers in 91 Patients

<table>
<thead>
<tr>
<th>Tumor Marker</th>
<th>AUC</th>
<th>( p )</th>
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<tbody>
<tr>
<td>NAB alone</td>
<td>0.928 (95% CI: 0.887–0.969)</td>
<td></td>
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<tr>
<td>NAB with tumor marker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYFRA (15.7)</td>
<td>0.993 (95% CI: 0.983–1.000)</td>
<td>0.001</td>
</tr>
<tr>
<td>CEA (0.6)</td>
<td>0.950 (95% CI: 0.913–0.988)</td>
<td>0.062</td>
</tr>
<tr>
<td>SCC (0.86)</td>
<td>0.957 (95% CI: 0.922–0.992)</td>
<td>0.057</td>
</tr>
<tr>
<td>CYFRA + CEA</td>
<td>0.993 (95% CI: 0.981–1.000)</td>
<td>0.001</td>
</tr>
<tr>
<td>CYFRA + SCC</td>
<td>0.995 (95% CI: 0.989–1.000)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CEA + SCC</td>
<td>0.966 (95% CI: 0.935–0.997)</td>
<td>0.023</td>
</tr>
<tr>
<td>CYFRA + CEA + SCC</td>
<td>0.995 (95% CI: 0.988–1.000)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Numbers in parentheses are cutoff levels of tumor markers in ng/ml.

Nine cases of nondiagnostic results were excluded from calculation of AUC.

AUC, areas under the curve; CI, confidence interval; CEA, carcinoembryonic antigen; SCC, squamous cell carcinoma; CYFRA, cytokeratin 19 fragments; NAB, needle aspiration biopsy.
Although CT-guided biopsy is a relatively safe and accurate method for diagnosing lung lesions, the results of transthoracic needle biopsy of lung lesions are often falsely negative in the diagnosis of malignancy, and nonspecific results are common in NAB. The reported incidence of nonspecific results ranges from 5.5 to 29%. Wieskopf et al. reported that serum CYFRA 21-1 and nonspecific results are common in NAB. The reported incidence of nonspecific results ranges from 5.5 to 29%. Wieskopf et al. reported that serum CYFRA 21-1 and nonspecific results are common in NAB. The reported incidence of nonspecific results ranges from 5.5 to 29%. Wieskopf et al. reported that serum CYFRA 21-1 and nonspecific results are common in NAB. The reported incidence of nonspecific results ranges from 5.5 to 29%. Wieskopf et al. reported that serum CYFRA 21-1 and nonspecific results are common in NAB. The reported incidence of nonspecific results ranges from 5.5 to 29%. Wieskopf et al. reported that serum CYFRA 21-1 and nonspecific results are common in NAB. The reported incidence of nonspecific results ranges from 5.5 to 29%. Wieskopf et al. reported that serum CYFRA 21-1 and nonspecific results are common in NAB. The reported incidence of nonspecific results ranges from 5.5 to 29%.

We used cytological fluid instead of blood serum as a new source of sample for tumor marker analysis. Although blood is a good sample for serum biomarkers and contains a large proteome that reflects a person’s health status, one of the main drawbacks of serum tumor markers is that they are typically only found in high concentrations near the tumor tissue or when the disease is at an advanced stage. Therefore, it remains very difficult to clinically detect a lung tumor at an early stage with serum marker assays. Cytoplasmatic fluid might be a more effective sample than blood for evaluation of tumor markers because it is obtained directly from NAB and, therefore, might contain many candidate biomarkers in high concentrations.

According to the results of our study, additional evaluation of tumor markers in the cytological fluid can improve the diagnostic performance of CT-guided NAB in patients with NSCLC. Our results showed a significant increase in overall sensitivity for NAB combined with a tumor marker compared with NAB alone (100% for CYFRA 21-1, 92.9% for CEA, and 94.2% for SCC; p = 0.001, p = 0.025 and p = 0.014, respectively). Furthermore, in subgroup analysis, the sensitivity also increased significantly for NAB combined with tumor marker results compared with NAB alone in stage 1 (100% for CYFRA 21-1, 89.5% for CEA, and 89.5% for SCC; p = 0.002, p = 0.040, and p = 0.040, respectively). By calculating ROC curves, we observed that the AUC of NAB with CYFRA 21-1 was significantly larger than the AUC of NAB alone, which indicates that this cytological tumor marker has additional value in the diagnosis of NSCLC.

In our study, the cytological marker CYFRA 21-1 had the best diagnostic performance, similar to findings of previous studies. Wieskopf et al. reported that serum CYFRA 21-1 was a sensitive and specific tumor marker for diagnosing NSCLC and seemed to be more sensitive and more specific than other tumor markers, such as CEA and SCC. Another study also showed that CYFRA 21-1 determination is useful in identification of early NSCLC. In previous reports, the combination of several tumor markers usually obtained better diagnostic performance than any one marker alone. Ferrer et al. reported that the combination of CYFRA 21-1, CEA, and CA 125 in either serum or pleural fluid improves the diagnostic value of pleural fluid cytology in the diagnosis of malignant pleural effusion. In their study, the combination of CYFRA 21-1, CEA, and CA 125 in pleural fluid achieved the best sensitivity (65%) with maximum specificity in the diagnosis of malignant pleural effusion. We also analyzed whether a combination of two or three tumor markers in the cytological fluid can have additional value in the diagnosis of NSCLC compared with a single tumor marker. The sensitivity and AUC of NAB with CYFRA 21-1 was not significantly different from that of NAB with any combination of tumor markers (p > 0.05). This finding probably reflects the high diagnostic value of CYFRA 21-1. Therefore, our results suggest that adding other tumor markers to CYFRA 21-1 has little additional value in terms of diagnostic performance for NSCLC.

Our study has some limitations. The number of cases involved was small, and the two major histological types were not represented in equal numbers as more than half of the cases included were ACs. Therefore, the value of cytological tumor markers shown in this study would be limited in cell types other than AC. Furthermore, the biopsied samples were predominantly malignant because we excluded cases that were most likely to be benign. Although NAB is a safe and effective test, it is also an invasive procedure for patients. Therefore, we deferred to follow-up imaging studies in cases with highly suspected benign lesions than NAB procedure.

Second, the results may be influenced by the method used for choosing the cutoff point for cytological tumor markers. To the best of our knowledge, no other study on the measurement of cytological tumor markers in patients with NSCLC has been published; therefore, there are no reference normal values for cytological fluid levels of various tumor markers. In our study, we used ROC curves to determine the cutoff values of tumor markers in the cytological fluid. The ROC curve, best accuracy, best specificity, 95% specificity, or using the same reference level as serum are all methods that could be used to select the cutoff level. As diagnostic tests require high specificity and sensitivity, we selected the cutoff level for each marker based on the best diagnostic accuracy. Nevertheless, because of the small sample size, we did not perform independent validation of the cutoff values selected for tumor markers as an independent series in this study. Third, although most lesions had histopathologically confirmed diagnoses, 30 malignant lesions were cytologically confirmed, and 13 lesions required follow-up imaging studies and clinical examinations.

In conclusion, measurement of the tumor markers CYFRA 21-1, CEA, and SCC in the cytological fluid can improve the diagnostic performance of CT-guided NAB for NSCLC, and of these, CYFRA 21-1 is the most useful cytological tumor marker. Analysis of tumor markers would be a helpful ancillary studies for the diagnosis of lung cancer. In cases where cytology tumor marker was positive, even if biopsy turned out to be negative, underlying malignancy can be suggested. Based on our study, cytological fluid seems to be a suitable sample for assessing the presence and concentrations of tumor markers and might be clinically useful in the diagnosis of lung cancer. This is particularly important as, although lung biopsy using needle aspiration is a confirmative method for diagnosis of lung cancer, NAB can give nondiagnostic results in up to 29% of cases. Performing the extra step of measuring concentrations of tumor markers in the...
fl uid that is aspirated does not require an additional puncture, takes little extra time, and is easy. Therefore, we believe that in cases of suspicious malignant nodules or masses showing a negative or inconclusive cytological result, evaluation of tumor markers in the cytological fluid may be a helpful complementary tool for the diagnosis of lung cancer.

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