CME/SAM

NSCLC Subtype Prediction Using Cytologic Fluid Specimens From Needle Aspiration Biopsies

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Key Words: Needle aspiration biopsy; Cytologic fluid; Serum; Non–small cell lung cancer; CYFRA 21-1; CEA; SCCA; Fluorine-18-fluorodeoxyglucose

DOI: 10.1309/AJCPYOJYG56UNBSZ

Upon completion of this activity you will be able to:
- describe the factors contributing to fluorine-18-fluorodeoxyglucose uptake in tumors as detected in positron electron tomographic/computed tomographic (PET/CT) scans.
- discuss tumor marker levels of squamous cell carcinoma antigen, cytokeratin 19, and carcinoembryonic antigen in non–small cell lung cancer subtypes.
- correlate PET/CT findings with serum levels and tumor cellular expression of selected tumor markers.

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The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

Questions appear on p 397. Exam is located at www.ascp.org/ajcpcme.

Abstract

This study evaluated the diagnostic usefulness of tumor marker concentrations in cytologic fluids (CF) for subtyping non–small cell lung cancer (NSCLC) and assessed the relationship between fluorine-18-fluorodeoxyglucose (^{18}F-FDG) uptake with serum and CF tumor marker levels. This prospective study included 88 patients diagnosed with adenocarcinoma or squamous cell carcinoma (SCC). Cytokeratin-19 fragment (CYFRA 21-1), carcinoembryonic antigen (CEA), and squamous cell carcinoma antigen (SCCA) concentrations in the CF samples were correlated with serum tumor marker concentrations, ^{18}F-FDG uptake, and NSCLC subtype.

Fifty-eight patients were diagnosed with adenocarcinoma. Multivariate analysis revealed higher CF and serum SCCA levels; smoking status predicted SCC from adenocarcinoma. CF SCCA showed the highest accuracy (83%) in distinguishing between SCC and adenocarcinoma. CF samples obtained during routine needle aspiration biopsy procedure contain tumor marker levels sufficient to distinguish between SCC and adenocarcinoma; CF SCCA had the highest diagnostic accuracy.

Lung cancer, the leading cause of cancer death worldwide, has a 5-year survival rate of less than 15%. However, recently developed chemotherapeutic agents and molecular targeting agents for lung cancer have significantly improved the prognosis for nonsquamous non–small cell lung cancer (NSCLC).[1-5] Accordingly, accurate subclassification of these NSCLCs becomes increasingly important.

Despite increasing public awareness of lung cancer and use of screening tests, fewer than 20% of patients newly diagnosed with lung cancer qualify for surgery. Thus, to optimize the use of available nonsurgical and palliative treatments, accurate diagnosis and subtyping are of paramount importance. The current established biopsy methods include computed tomography (CT)– or fluoroscopy-guided transthoracic needle aspiration biopsy (NAB) and transbronchial needle aspiration. Although these methods provide satisfactory diagnostic accuracy for malignancy, the reported accuracy for NSCLC subtyping varies.[6-8] To increase subtyping accuracy in NSCLC, methods using serum tumor markers[9] and immunohistochemical staining of biopsy specimens have been investigated.[10,11]

Factors that significantly reduce accuracy of diagnosis and subtyping of NSCLC include small sample size and tumor heterogeneity. In large necrotic tumors, necrotic debris may be aspirated in higher proportion than viable tumor cells. The 3 tumor markers most often used clinically are squamous cell carcinoma antigen (SCCA), cytokeratin-19 fragment (CYFRA 21-1), and carcinoembryonic antigen (CEA). Because these markers originate in tumor cells, fluids directly aspirated from the tumor may contain higher tumor marker

concentrations than the serum, and these cytologic fluid (CF) markers may reflect tumor histopathology more accurately than those of serum tumor markers. We hypothesized that analysis of tumor markers in fluid specimens obtained by aspiration would increase the accuracy in subtyping NSCLC.

Fluorine-18-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) is currently well integrated into the diagnostic workup for lung cancer. The ^{18}F-FDG uptake in the primary lung malignancy correlates positively with tumor size,[12] glucose transporter-1,[13] and Ki-67 index (MIB-1 staining, proliferation marker)[14] and negatively with prognosis.[15,16] Studies also correlate ^{18}F-FDG uptake with histologic grading[17] because ^{18}F-FDG uptake is lower in adenocarcinoma than in squamous cell carcinoma (SCC) and small cell carcinoma.[18] However, no studies to date have shown the correlation between tumor marker levels with ^{18}F-FDG uptake in patients with lung cancer.

We conducted this study to evaluate the diagnostic usefulness of CF tumor markers for NSCLC subtyping and to assess the relationship between ^{18}F-FDG uptake in the primary lesion with serum and CF tumor marker levels.

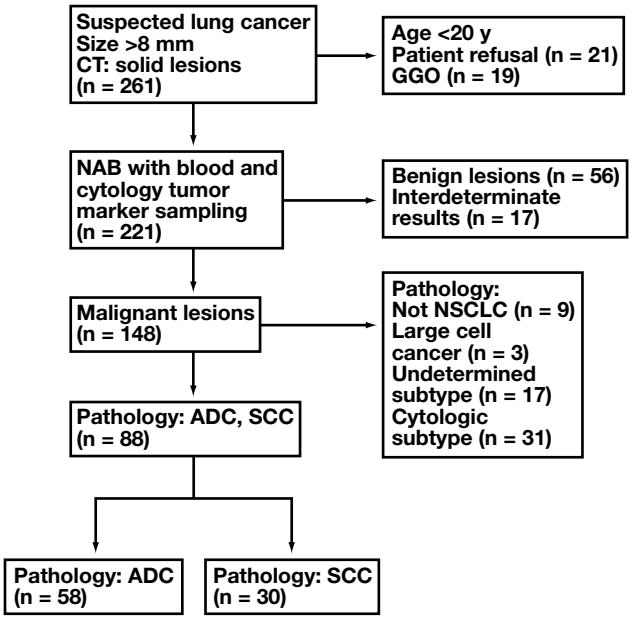

Figure 1 Overview of the study design. ADC, adenocarcinoma; CT, computed tomography; GGO, ground glass opacity; NAB, needle aspiration biopsy; NSCLC, non–small cell lung cancer; SCC, squamous cell carcinoma.

Materials and Methods

Patient Selection

The 261 patients enrolled in this prospective study underwent transthoracic NAB for the differential diagnosis of a pulmonary nodule or mass on CT during the interval from November 1, 2009, to July 31, 2010. Patients met inclusion criteria if they were more than 20 years of age and had a solid lesion, which was defined as a lesion greater than 8 mm with ground glass opacity component less than 50%. Patients were excluded if they had lesions with ground glass opacity (n = 19) or refused to provide written informed consent (n = 21). Of these 221 patients, 148 patients had malignant lesions, 56 had benign lesions, and 17 had indeterminate results. To test the application of CF tumor marker levels in subtyping NSCLCs, only patients with lesions pathologically confirmed to be adenocarcinoma or SCC were included (9 patients with small cell lung cancer or lymphoma, 3 with large cell carcinoma, 17 with NSCLC of undetermined subtype, and 31 with cytologically confirmed NSCLC subtype were excluded). Finally, 88 patients (64 men and 24 women; average age, 66.4 years; range, 39-87 years) who had pathologically confirmed adenocarcinoma or SCC were included. All patients underwent routine ^{18}F-FDG PET/CT within 10 days of biopsy to evaluate for distant metastasis before further therapy. The patient selection process is summarized in **Figure 1**.

Data collection was systematized and a standardized registration form was prepared. The institutional review board approved the study protocol, and all patients provided written informed consent.

Percutaneous Transthoracic NAB Technique

The biopsies were performed by 3 chest radiologists who had 4, 6, and 10 years of experience performing thoracic biopsies. Fluoroscopy-guided biopsy interventions (n = 20) were performed using a Medix 130 fluoroscope (Hitachi Medical Corporation, Tokyo, Japan), and CT-guided biopsy interventions (n = 68) were performed using a 16-MDCT scanner (Somatom Sensation 16, Siemens Medical Solutions, Malvern, PA) equipped with CARE Vision software (Siemens). During each procedure, more than 2 aspiration specimens were obtained using 20- to 22-gauge Chiba needles connected to a 10-mL syringe without additional needle punctures. Part of each aspirate was spread onto glass slides and smears prepared for cytologic examination; another part of the material was prepared in a tube for cell block processing. All smears were immediately placed in 95% ethanol for Papanicolaou (Pap) staining. The remainder of each aspirate (1-2 mL) was rinsed with 1 mL of normal saline solution in a tube for evaluating cytologic tumor markers.

Tumor Marker Analysis

Blood and CF specimens were collected from each patient before beginning any therapy. Serum and CF supernatants were obtained by centrifugation at 2,000*g* for 10 minutes

DOI: 10.1309/AJCPYOJYG56UNBSZ

and stored at –40°C before performing tumor marker assays using commercial immunoassay kits. Technicians performing assays of both CF and serum samples were blinded to the corresponding diagnoses. CYFRA 21-1 levels were measured using an electrochemiluminescent immunoassay (CYFRA 21-1, Roche Diagnostics, Mannheim, Germany), CEA levels were measured using a chemiluminescence immunoassay (Centaur CEA, Bayer HealthCare, Tarrytown, NY), and SCCA levels were measured using an immunoradiometric assay (SCC-RIABEAD, SRL, Tokyo, Japan). Tumor markers in each of the CF samples were evaluated twice, and the mean values were used for analysis. Detectable levels for each CF tumor marker were defined as follows: 0.1 to 500 for CYFRA 21-1; 0.1 to 500 for CEA; and 0.01 to 150 for SCCA.

Histologic Analysis

The CF specimen from the NAB was fixed in 95% ethanol and stained using the Pap method. The tumor tissue sample collected during the operation was imprinted on a glass slide. Tissue sections were processed for H&E staining. Histologic classification was done according to the proposed International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society international multidisciplinary classification of lung adenocarcinoma. If there was a disagreement in the histopathologic analysis, a consensus was achieved by a joint reading.

PET/CT Protocol

All patients underwent routine ^{18}F-FDG PET/CT scanning with DSTe PET/CT (GE Healthcare, Milwaukee, WI). All patients fasted for at least 6 hours, and glucose levels in peripheral blood in all patients were confirmed to be 140 mg/dL (7.8 mmol/L) or less before ^{18}F-FDG injection. Approximately 5.5 MBq/kg of body weight of ^{18}F-FDG was administered intravenously 1 hour before image acquisition. After the initial low-dose CT, a standard PET imaging scan from the neck to the proximal thighs with an acquisition time of 3 minutes/bed in 3-dimensional mode was performed.

Imaging Analysis

One experienced nuclear medicine specialist who was blind to the clinical information interpreted the PET images qualitatively by visual inspection. Visual analysis was performed at a GE AW 4.0 workstation. Semiquantitative analysis was performed by drawing a region of interest on the whole lung mass that was aspirated, and the maximum standard uptake value (SUV) was obtained.

Statistical Analysis

Differences between the adenocarcinoma and SCC groups were evaluated using the Fisher exact test (sex, T stage, TNM stage) or *t* test (CF and blood tumor marker levels, SUV, age, tumor size). A paired *t* test was performed to compare each patient's tumor marker levels in CF with those in serum. Correlations between CF and serum tumor markers, SUV, age, and tumor size were performed using the Pearson correlation analysis. Student *t* test was used to compare CF and serum tumor marker levels and SUV with the absence or presence of distant metastases. To predict NSCLC subtype, pathology results were used as a reference standard. Univariate analysis was performed to evaluate for significant correlations in CF and serum tumor marker levels, PET SUV, as well as other clinical data such as patient age, tumor size, gender, T stage, and TNM stage. Statistically significant findings on univariate analysis were used in multivariate analysis.

Receiver operating characteristic (ROC) curves were constructed using the CF and serum tumor marker values. To compare the performance of CF and serum tumor markers, the areas under the curves (AUCs) were compared using the DeLong method for statistical significance.[19] A cutoff value was determined for the optimal differentiation between adenocarcinoma and SCC. The cutoff level selected for each marker was based on the best diagnostic accuracy. The accuracy of tumor markers was compared using the generalized estimating equation method. Statistical analyses were performed with SAS software (version 9.2 for Windows; SAS Institute, Cary, NC), and *P* values less than .05 were considered statistically significant.

Results

Patient Demographics

Of the 261 patients initially identified, 88 patients (34%) had a pathologically confirmed subtype of adenocarcinoma (n = 58) or SCC (n = 30) and underwent PET/CT before further therapy. Of these 88 patients, 46 had histologic confirmation either with a lobectomy (n = 45) or an excisional biopsy (n = 1), and the remaining patients had histologic confirmation on transbronchial or NAB of the lung. **Table 1** summarizes characteristics of the patients included in our study. We divided the smoking history into former (defined as more than 3 months of smoking cessation before lung cancer diagnosis), current smokers, and never smokers. In accordance with previous reports,[20,21] a history of never smoking was significantly more prevalent among patients with adenocarcinoma than among those with SCC. Former and current smokers were also subcategorized according to pack-years and smoking cessation history and compared with NSCLC subtype prediction, but this did not achieve statistical significance, more likely because of the small number of patients in each group (data not shown). The patients with adenocarcinoma included

DOI: 10.1309/AJCPYOJYG56UNBSZ

Table 1
Baseline Patient Characteristics and Analysis of Variables Distinguishing Between ADC and SCC

Variable	Mean ± SD ADC (n = 58)	Mean ± SD SCC (n=30)	*P*
Age, y	65.3 ± 10.34	68.5 ± 6.84	.083
Sex (F/M)	23/35	1/29	<.001[a]
Lesion size, mm	30.1 ± 20.26	32.0 ± 17.00	.664
Smoking status, No. (%)			.011[b]
Former	15 (17)	13 (15)	
Current	10 (11)	10 (11)	
Never	33 (38)	7 (8)	
Hypertension	21	10	.820
Diabetes mellitus	9	6	.770
Tuberculosis	4	5	.260
Tumor stage			
T stage (1/2/3/4)	21/16/9/12	14/4/4/8	.458
Stage (1/2/3/4)	22/5/7/24	15/2/5/8	.491
Tumor marker			
Serum SCCA	0.9 ± 0.87	1.6± 1.70	.014[a]
Serum CYFRA 21-1	11.7 ± 40.10	4.6 ± 5.23	.336
Serum CEA	52.0 ± 114.70	4.4 ± 4.99	.003[a]
CF SCCA	5.8 ± 18.47	59.3 ± 61.45	<.001[a]
CF CYFRA 21-1	113.9 ± 158.59	164.6 ± 194.58	.224
CF CEA	31.8 ± 94.50	20.0 ± 34.93	.512
SUV	7.0 ± 5.27	9.9 ± 5.59	.024[a]

ADC, adenocarcinoma; CEA, carcinoembryonic antigen; CF, cytologic fluid; CYFRA 21-1, cytokeratin-19 fragment; SCC, squamous cell carcinoma; SCCA, squamous cell carcinoma antigen; SD, standard deviation; SUV, standard uptake value.
[a] Statistically significant ($P < .05$).
[b] Statistically significant ($P < .05$) compared with never smokers.

significantly more women, but this was no longer seen after adjusting for smoking history.

Comparison of CF and Serum Tumor Markers

Concentrations of CYFRA 21-1 were significantly higher in the CF samples (mean ± SD, 131.2 ± 172.28; range, 0.95-500.0) than in serum (mean ± SD, 9.3 ± 32.77; range, 0.68-288.0; $P < .0001$). CEA levels were higher in serum (mean ± SD, 35.8 ± 95.63; range, 0.7-500.0) compared with CF (mean ± SD, 27.8 ± 79.30; range, 0.1-500.0), but the differences were not statistically significant ($P = .467$). SCCA concentrations in CF (mean ± SD, 24.0 ± 46.18; range, 0.07-150.0) were significantly higher than those in serum SCCA (mean ± SD, 1.1 ± 1.25; range, 0.1-9.2; $P < .001$). Pearson correlation data for CF tumor markers are shown in **Table 2**.

Elevated serum CEA levels reportedly indicate the presence of distant metastasis.[22,23] Of our 88 patients, 32 showed distant metastasis on PET/CT scans. Patients in the stage IV group had significantly higher serum CEA levels compared with patients in the stage I-III group (mean ± SD, 86.4 ± 144.60 vs 6.9 ± 18.18; $P = .004$). SUV for the primary lesion was also higher in the stage IV group than in the stage I-III group (mean ± SD, 9.6 ± 5.67 vs 7.1 ± 5.26; $P = .042$). No other serum tumor markers or CF tumor markers were significantly different between the stage I-III and stage IV groups.

Prediction of NSCLC Subtype

Analysis of clinical factors and tumor marker levels in predicting NSCLC subtype are shown in Table 1. Univariate analysis showed that CF SCCA, serum SCCA, serum CEA, SUV, smoking status, and sex predicted SCC over adenocarcinoma. The significant factors on univariate analysis were used to predict NSCLC subtype in multivariate analysis **Table 3**. Several multivariate models were constructed to minimize the effects of confounding factors. Model 1 evaluated the strength of association between CF SCCA and serum CEA in distinguishing between SCC and adenocarcinoma. This model showed that the predictive value of serum CEA for SCC was lost when CF SCCA was considered. Model 2 reveals that both CF SCCA and serum SCCA predict SCC from adenocarcinoma. Considering the relationship between SUV and tumor size, model 3 was used to evaluate whether SUV can predict SCC when tumor size is controlled. Model 4 was used to show that, although SUV could predict SCC better than it did adenocarcinoma, CF SCCA was a stronger predictor of SCC. When we correlated smoking status with other clinical and tumor markers, we found that smoking status independently predicted

Table 2
Univariate Analysis of Variables Correlated With Tumor Markers in Cytologic Fluid and Serum

	CF SCCA		CF CEA		CF CYFRA 21-1	
Variable	Pearson Coefficient	*P*	Pearson Coefficient	*P*	Pearson coefficient	*P*
SUV	0.295	.005[a]	–0.005	.961	0.067	.535
Size	0.008	.942	0.075	.489	0.125	.248
Age	0.045	.677	0.032	.767	–0.056	.601
Serum SCC	0.106	.324	0.033	.757	0.042	.696
Serum CYFRA 21-1	–0.079	.466	–0.021	.846	0.221	.038[a]
Serum CEA	–0.168	.118	0.324	.002[a]	0.158	.142

CEA, carcinoembryonic antigen; CF, cytologic fluid; CYFRA 21-1, cytokeratin-19 fragment; SCC, squamous cell carcinoma; SCCA, squamous cell carcinoma antigen; SUV, standard uptake value.
[a] Statistically significant ($P < .05$).

DOI: 10.1309/AJCPYOJYG56UNBSZ

SCC. We included smoking status in model 5, and this final model identified smoking status, serum SCCA, and CF SCCA as the strongest predictors of SCC pathology.

Areas under the ROC curves are compared in **Figure 2**. The AUC for CF SCCA (0.86) was significantly larger than any other CF or serum tumor markers. The highest cutoff value for each tumor marker was evaluated using ROC analysis. **Table 4** shows the sensitivity, specificity, positive and negative predictive values, and accuracy of each CF and serum tumor marker in subtyping NSCLC as adenocarcinoma or SCC using the cutoff values obtained from ROC analysis. Among the tumor markers, CF SCCA showed the highest accuracy, which was significantly higher than serum SCCA (83% vs 63.6%; $P = .005$). The accuracy of serum CEA was significantly higher than that of CF CEA (55.7% vs 28.2%; $P = .002$). Serum and CF CYFRA 21-1 did not differ significantly in accuracy (47.7% vs 48.9%; $P = .857$).

Table 3
Multivariate Analysis Models to Distinguish Between Adenocarcinoma and Squamous Cell Carcinoma

	Odds Ratio	95% CI	*P*
Model 1			
CF SCCA	0.005	0.004-0.007	<.001[a]
Serum CEA	0.000	–0.002-0.000	.104
Model 2			
CF SCCA	0.005	0.004-0.007	<.001[a]
Serum SCCA	0.078	0.011-0.144	.023[a]
Model 3			
SUV	0.023	0.004-0.042	.042[a]
Size	–0.001	–0.007-0.004	.644
Model 4			
CF SCCA	0.005	0.003-0.007	<.001[a]
SUV	0.008	–0.008-0.024	.332
Model 5			
CF SCCA	0.005	0.003-0.007	<.001[a]
Serum SCCA	0.077	0.011-0.142	.022[a]
SUV	0.007	–0.008-0.023	.348
Smoking status	0.189	0.011-0.349	.037[a]

CEA, carcinoembryonic antigen; CF, cytologic fluid; CI, confidence interval; SCCA, squamous cell carcinoma antigen; SUV, standard uptake value
[a] Statistically significant ($P < .05$).

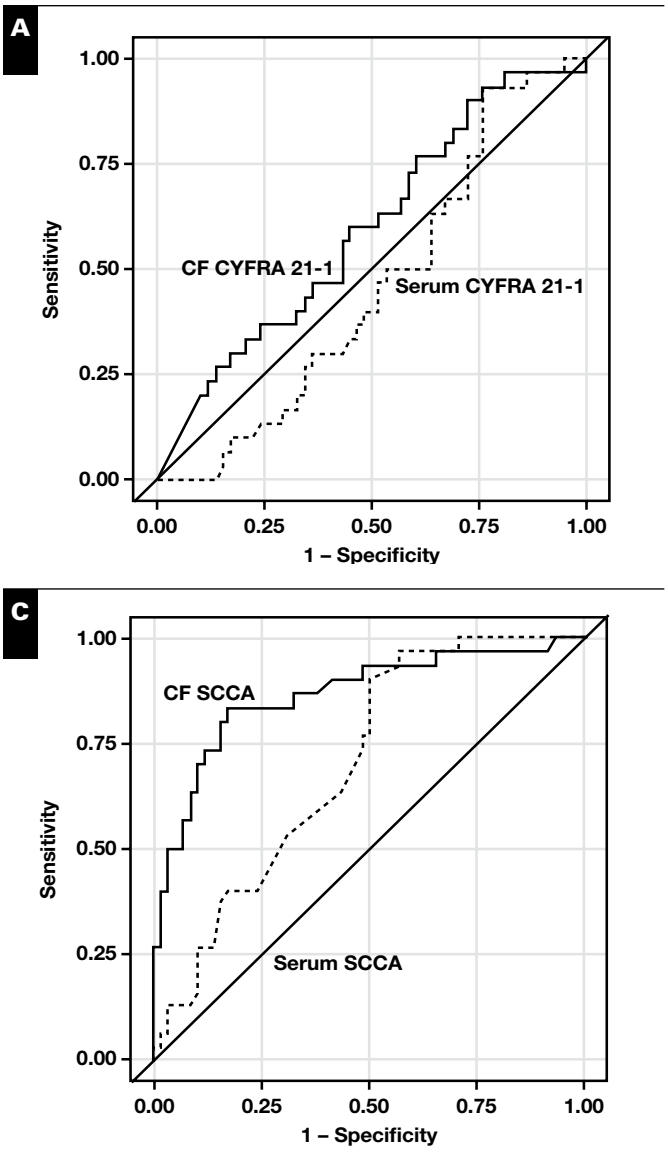

Figure 2 Receiver operating characteristic analyses comparing serum tumor markers with cytologic fluid (CF) tumor markers in distinguishing squamous cell carcinoma from adenocarcinoma. **A**, CYFRA 21-1 (area under the curve [AUC] for CF, 0.59; serum, 0.46; $P = .166$). **B**, CEA (AUC for CF, 0.51; serum, 0.59; $P = .201$). **C**, SCCA (AUC for CF, 0.86; serum, 0.70; $P = .011$). *P* values indicate significance of the AUC comparisons. CYFRA 21-1, cytokeratin-19 fragment; CEA, carcinoembryonic antigen; SCCA, squamous cell carcinoma antigen.

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Table 4
Sensitivity, Specificity, Positive and Negative Predictive Values, and Accuracy of Markers for NSCLC Subtyping

			Predictive Values		
	Sensitivity, No. (%)	Specificity, No. (%)	Positive, No. (%)	Negative, No. (%)	Accuracy, No. (%)
Cytologic fluid[a]					
CYFRA 21-1 (11.25 ng/mL)	27/30 (90.0)	16/58 (27.6)	27/69 (39.1)	16/19 (84.2)	43/88 (48.9)
CEA (0.27 ng/mL)	23/30 (76.7)	8/58 (13.8)	23/73 (31.5)	8/15 (53.3)	31/88 (35.2)
SCCA (5.65 ng/mL)	25/30 (83.3)	48/58 (82.8)	25/35 (71.4)	48/53 (90.6)	73/88 (83.0)
Serum[a]					
CYFRA 21-1 (6.51 ng/mL)	28/30 (93.3)	14/58 (24.1)	28/72 (38.9)	14/16 (87.5)	42/88 (47.7)
CEA (8.45 ng/mL)	28/30 (93.3)	21/58 (36.2)	28/65 (43.1)	21/23 (91.3)	49/88 (55.7)
SCCA (0.63 ng/mL)	27/30 (90.0)	29/58 (50.0)	27/56 (48.2)	29/32 (90.6)	56/88 (63.6)

CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin-19 fragment; NSCLC, non–small cell lung cancer; SCCA, squamous cell carcinoma antigen.
[a] Values in parentheses indicate the cutoff values obtained from receiver operating characteristic analysis (see text).

Discussion

We have shown in this study that the CF specimens obtained during routine NAB procedures contain tumor markers potentially useful in subtyping NSCLC. Our results indicate that high levels of SCCA obtained in CF specimens during NAB provide a sensitive and specific marker for SCC as distinguished from adenocarcinoma and that CF SCCA performs better than other serum tumor markers in this context. The present findings support previous findings that the tumor marker levels in CF may facilitate the subtyping, as well as diagnosis, of NSCLC.[24]

Serum and Cytologic Tumor Marker Correlation

An additional finding in our study was the correlation between tumor marker levels in CF and serum specimens. In particular, we found that CF specimens acquired during NAB contained significantly higher concentrations of CYFRA 21-1 and SCCA than the corresponding serum samples. The CEA levels tended to be higher in serum than in CF, but this most likely reflects higher levels of CEA in patients with metastases.[22,23]

The tumor marker concentrations in CF exceeded those in serum, but these correlations were weak or undetectable. Although CYFRA 21-1 concentrations were higher in CF than in serum CYFRA 21-1, the correlation between these 2 markers was weak. This suggests that the factors that govern CYFRA 21-1 release into the blood do not depend directly on CYFRA 21-1 levels in the tumor. In vitro studies show that serum CYFRA21-1 may reflect cell necrosis[25-27] and that caspase 3, a protease involved in apoptosis, plays a role in CYFRA 21-1 formation. Thus, serum CYFRA21-1 alone may not be sufficient to detect viable tumor cells, especially when the tumor is relatively small, with proportionally smaller necrotic regions. Previous studies using immunohistochemical analysis showed that cytokeratin-19, the marker used for CYFRA21-1, stained both adenocarcinoma and SCC strongly and indiscriminately.[28-30] These results and our own support the conclusion that CYFRA 21-1 obtained directly from the tumor will not predict NSCLC subtype any more accurately than serum CYFRA21-1.

We found that CF SCCA levels were 10-fold higher than serum SCCA levels but observed no direct correlation between SCCA levels in CF and serum. The serine proteinase inhibitor SCCA is a cytoplasmic component of normal squamous epithelial cells not usually detected in the serum. In tumor cells, SCCA resides in the cytosol, and its presence in the serum samples of patients with NSCLC may occur through passive release from cells.[31,32] This may explain the lack of correlation between serum and CF SCCA levels. This also suggests that direct aspirates of the tumor will contain markedly higher levels of SCCA than serum SCCA, which may translate into higher sensitivity for SCC diagnosis.

Production of NSCLC Subtype

Our results identified CF SCCA, from among all of the CF and serum tumor markers tested, as the one that distinguished between adenocarcinoma and squamous cell carcinoma with the greatest accuracy. The CF SCCA showed comparable sensitivity and superior specificity to serum SCCA in NSCLC subtyping. Previous reports show that at a cutoff value of 2 ng/mL, serum SCCA is very specific (0.95) but has low sensitivity (0.32) in distinguishing SCC from benign lesions.[23,33] Other studies report that serum SCCA is specific but not sensitive in differentiating SCC from adenocarcinoma.[34,35] We used the cutoff values obtained from the ROC curve analysis for both serum and CF tumor markers to compare the sensitivity, specificity, and accuracy of these markers under the same conditions. Using the ROC-generated cutoff value of 0.63 ng/mL, our results showed that serum SCCA had higher sensitivity (90%) and lower specificity (50%) for distinguishing SCC from adenocarcinoma compared with other studies. This may occur because the ROC analysis showed an increase in accuracy due to increased sensitivity rather than specificity. In contrast, the serum SCCA value

DOI: 10.1309/AJCPYOJYG56UNBSZ

used in routine lung cancer screening is set higher to increase the specificity in distinguishing benign from malignant lung lesions. Considering that CF SCCA showed high sensitivity (83%) and specificity (83%) and higher accuracy (83%) than any other tumor marker in the same population, the clinical benefit of using CF analysis for NSCLC subtyping is evident.

Previous studies correlated serum tumor markers with patient survival. Therefore, we sought to correlate SUV of the primary lesion with both CF and serum tumor markers. Clinical studies show that primary tumor ^{18}F-FDG uptake is correlated with patient prognosis,[17,18] and a recent meta-analysis supported the prognostic value of primary tumor SUV in NSCLC.[16] Our results support previous findings that ^{18}F-FDG uptake in the primary lesion is correlated with histology, tumor size, and tumor stage (stage I-III vs stage IV). We have also shown that CF SCCA with ^{18}F-FDG uptake is mildly correlated in the primary lesion and that serum tumor marker levels do not correlate with ^{18}F-FDG uptake. Other studies also show no correlation between serum tumor markers and SUV in other cancers.[36]

Limitations

The first limitation of our study was the selection of a higher proportion of patients with adenocarcinoma over SCC compared with reported ratios of lung cancer subtypes. This resulted from the selection bias inherent in NAB, which is the procedure recommended for peripheral lesions. Despite this bias, we showed that SCCA concentrations in CF specimens obtained from peripherally accessible lesions are sufficient for accurate subtyping.

A second limitation was that the results may have been influenced by the method used to set the cutoff values for tumor marker concentrations. In the absence of reference values for tumor markers that distinguish between SCC and adenocarcinoma, we conducted an ROC analysis using values for CF and serum tumor markers. By comparing ROC curves, we determined cutoff values for optimal NSCLC subtyping.

In summary, CF specimens obtained during routine NAB procedures contain tumor markers in concentrations sufficient to distinguish SCC from adenocarcinoma. The CF SCCA showed higher accuracy in this application than serum tumor markers. In addition, we found that ^{18}F-FDG uptake in NSCLC correlated only with CF SCCA levels and not with serum tumor marker levels. Thus, in cases in which cytologic findings are inconclusive for NSCLC subtyping, analysis of CF tumor markers may facilitate the decision.

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This research was supported by the Basic Science Research Program through the National Research Foundation of Korea, which is funded by the Ministry of Education, Science and Technology (2010-0009053).

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