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A Comparison of Serum CYFRA 21-1 and SCC Ag in the Diagnosis of Squamous Cell Lung Carcinoma

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To evaluate the usefulness of CYFRA 21-1 and SCC Ag in the diagnosis of squamous cell carcinoma (SQC) of the lung, we tested sera from 124 patients with lung cancers (squamous cell ca 72, adenoca 22, large cell ca 4, small cell ca 18 and undetermined 8) and 78 patients with inflammatory lung diseases (bronchitis 24, bronchiectasis 29, tuberculosis 19 and others 6) using immunoradiometric assay kit for cytokeratin fragment 19(CYFRA 21-1) and radioimmunoassay kit for SCC Ag. The serum CYFRA 21-1 and SCC Ag were significantly higher in lung cancer patients compared with control subjects. However, the significant difference was restricted only to SQC. In patients with SQC, CYFRA 21-1 and SCC Ag showed significantly higher levels according to the advanced anatomic stages (stage I-IIIa vs. stage IIIb, IV, $p<0.05$). There was a good correlation between CYFRA 21-1 and SCC Ag($r=0.41$, $p<0.001$). Receiver operating characteristic (ROC) curves were generated from results of both tumor markers and areas under the curves (AUC) were calculated. AUC of CYFRA 21-1(0.93) were significantly larger than that of SCC Ag (0.77) for the diagnosis of SQC ($p<0.05$). Therefore, we conclude that CYFRA 21-1 is superior to SCC Ag in the diagnosis of squamous cell carcinoma of the lung.

Key Words : *Squamous cell lung carcinoma, CYFRA 21-1, Cytokeratin, SCC Ag, Tumor marker*

INTRODUCTION

Several tumor markers, including CEA and SCC Ag, have been used as indices of disease extent, prognosis and response to therapy for patients with lung cancer. However, the utility of tumor markers for the carcinoma of the lung has been limited by the lack of sufficient sensitivity or specificity.

Squamous cell carcinoma antigen(SCC Ag) was developed from uterine cervical carcinoma[1] and it has been used for disease monitoring after therapy for uterine cervical squamous cell carcinoma[2]. However, this marker has also been reported to be useful for the squamous cell carcinoma of the lung[3-5].

Cytokeratins are expressed by all epithelial cells and the expression of cytokeratins remains during malignant transformation[6]. As

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Abbreviations :
NSCLC=non-small cell lung carcinoma
SQC=squamous cell carcinoma
ADC=adenocarcinoma
SCC=small cell carcinoma
LCC=large cell carcinoma
SCC Ag=squamous cell carcinoma antigen
CEA=carcinoembryonic antigen
NSE=neuron specific enolase
ROC=receiver operating characteristics
AUC=Area under the curve

Table 1. Characteristics of Control Subjects and Patients with Lung Cancer

Group (number)	Control (78)	Lung Cancer (124)
Age (mean (SD))	58.3 (12.4)	62.0 (8.9)
Sex (M/F)	50/28	101/23
Smoking (yes/no)	42/36	92/32
Pack-years (mean (SD))	15.8 (19.3)	31.3 (22.3)
Type	bronchitis (24)	SQC (72)
	bronchiectasis (29)	ADC (22)
	tuberculosis (19)	LCC (4)
	others (6)	SCC (18)
		undetermined (8)

SQC : squamous cell carcinoma, ADC : adenocarcinoma, LCC : large cell carcinoma
SCC : small cell carcinoma, SD : standard deviation

the cytokeratins might be released into the serum, owing to cell lysis and tumor necrosis, the significance of serum cytokeratin fragment in lung cancer has been studied previously[7-12]. In those reports, serum cytokeratin fragments have been regarded as a useful diagnostic tool, especially for squamous cell carcinoma of the lung, and also as an independent prognostic variable.

The objective of this study was to evaluate the diagnostic usefulness of CYFRA 21-1 and SCC Ag and to compare their value for the diagnosis of lung carcinoma.

MATERIAL AND METHODS

1. Subjects

We collected 202 serum samples from those who were referred to our laboratory for bronchoscopic examinations from January 1993 to December 1994. After the final diagnoses were made, data was reviewed and subjects were grouped into cancer and non-cancer groups, retrospectively. Of the 202 patients, 124 were diagnosed having lung cancer, 72 squamous cell carcinoma, 22 adenocarcinoma, 4 large cell carcinoma, 18 small cell carcinoma and 8 undetermined type of lung carcinoma. Of seventy-two patients with squamous cell carcioma, 4 patients were in stage I, 5 in stage II, 28 in stage IIIa, 28 in stage IIIb and 7 in stage IV. Histologic classification and anatomic staging were based on the World Health Organization report[13] and the new international staging system for lung cancer[14]. The 78 patients, who were grouped as controls, had tuberculosis, pneumonia and chronic obstructive pulmonary disease (Table 1).

2. Assay

For the detection of cytokeratin fragment 19, CYFRA 21-1 immunoradiometric assay kits (Cis Bio International, Gif/Yvette, France) were used. Serum samples had been deep frozen until tested. Two mice monoclonal antibodies obtained from MCF7 cell line were used for this two site sandwich method. The sera of patients were incubated in polystyrene spheres coated with monoclonal antibody KS 19-1 for 20 hours at 2~8℃, then washed with distilled water and incubated in ^{125}I-labeled BM 19-21 for 3 hours at 2~8℃. After washing the sphres once again with distilled water, radioactivity was detected in a well-type gamma counter. A standard curve was obtained by plotting the amount of the bound radioactivity versus the cytokeratin concentrations of the standards. Then the radioactivity, expressed as cpm, was converted to ng/ml using the standard curve.

For the detection of squamous cell carcinoma antigen(SCC Ag), we used SCC RIA-BEAD Kit (Abbott Laboratories, Abbott Park, IL, USA) which is also a kind of the two-site sandwich method. The testing method was not different from CYFRA 21-1, except using the beads coated with anti-SCC Ag mouse monoclonal antibodies.

Table 2. Comparison of CYFRA 21-1 and SCC Ag between Control and Lung Cancer Groups

	CYFRA 21-1 (ng/ml)		SCC Ag (ng/ml)	
	median value	interquartile range	median value	interquartile range
Control (78)	1.6	1.0 - 2.5	1.5	0.7 - 2.2
Cancer (124)	5.3	2.4 - 15.8*	2.3	1.1 - 4.7*
NSCLC (98)	6.2	2.9 - 18.9	2.5	1.4 - 5.5
SQC (72)	7.4	3.2 - 20.4*	3.1	1.7 - 6.2*
ADC (22)	4.3	2.4 - 11.5	2.1	1.1 - 2.6
SCC (18)	2.7	1.5 - 5.8	1.5	0.8 - 2.4

* p<0.001
※ Comparisons were made between control subjects and each histologic group of lung cancer

Table 3. Distribution of Diagnostic Values for the Detection of SQC with Varying Cut-Off Values

cut-off value	sensitivity	specificity	PPV*
CYFRA 21-1			
5.3 ng/ml	58.6%	95.9%	93.2%
3.3 ng/ml	70.0%	87.7%	84.5%
1.0 ng/ml	97.1%	31.5%	57.6%
SCC Ag			
3.9 ng/ml	44.3%	95.9%	91.2%
1.0 ng/ml	85.7%	41.5%	55.0%
0.7 ng/ml	94.3%	16.6%	53.4%

* PPV : positive predictive value

3. Statistical Analysis

The values of both tumor markers were expressed in the median and interquartile range. We used the SAS statistical analysis package (SAS Institute, Cary, N.C. USA) for the frequency tables, t-test, univariate analysis and non-parametric tests. ROC (receiver operating characteristics) curves were plotted with varying degree of cut-off values for CYFRA 21-1 and SCC Ag to compare their diagnostic sensitivity. Areas under the ROC curves were calculated and compared as reported previously[15, 16].

RESULTS

CYFRA 21-1 and SCC Ag both showed significant differences between normal control and cancer groups. The median values and interquartile ranges of serum CYFRA 21-1 and SCC Ag were significantly higher in lung cancer patients (CYFRA 21-1:5.3, 2.4~15.8, SCC Ag:2.3, 1.1~4.7) compared with control subjects (CYFRA 21-1:1.6, 1.0~2.5, SCC Ag:1.5, 0.7~2.2). Both markers showed significantly higher levels in squamous cell carcinoma compared to controls. However, there were no statistically significant differences between other cell types of lung cancer and controls (Table 2). Therefore, we present the following results only for cases with squamous cell carcinoma.

In patients with squamous cell carcinoma, CYFRA 21-1 and SCC Ag, both showed significantly higher levels according to the advanced anatomic stages (stage I-IIIa vs. stage IIIb, IV), although there were no differences in comparison of individual stages (Fig. 1 and Fig. 2).

In comparing the relationship between CYFRA 21-1 and SCC Ag, there was a good correlation between both tumor markers (N= 150, r=0.41, p<0.001). However, as shown in Fig. 3, CYFRA 21-1 was more dispersed than SCC Ag between the control and squamous

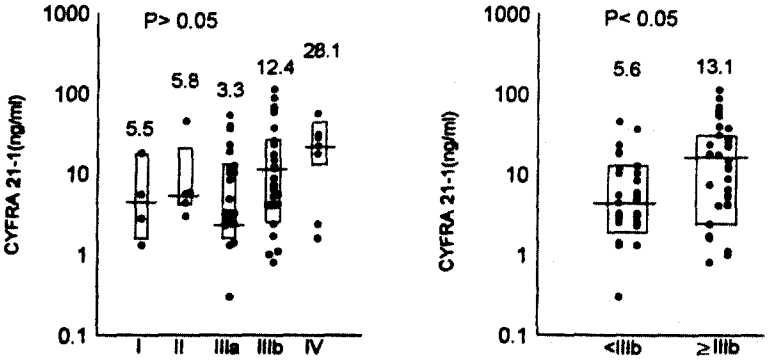

Fig. 1. Distribution of CYFRA 21-1 according to the anatomic stage in cases with squamous cell lung cancer (— : median value, columns : interquatile range).

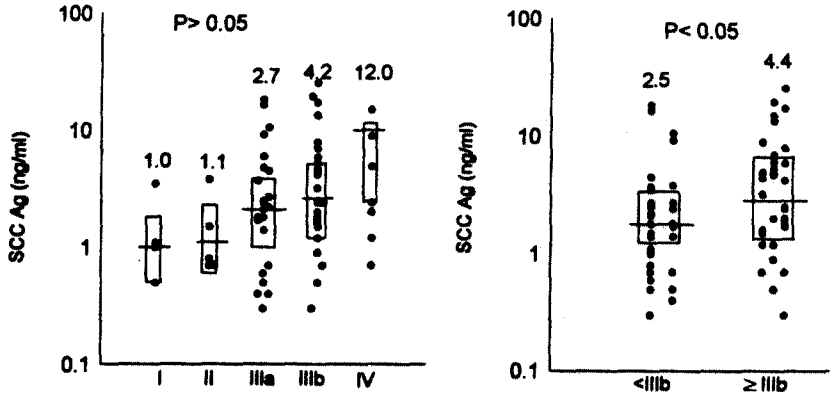

Fig. 2. Distribution of SCC Ag according to the anatomic stage in cases with squamous cell lung cancer (— : median value, columns : interquatile range).

cell carcinoma groups.

When we used SCC Ag, with the cut-off value of 3.9 ng/ml, sensitivity was only 44.3%, and the specificity was 95.9%. With the cut-off value of 1.0 ng/ml, sensitivity increased to 85.7% with a marked loss of specificity to 41.5%. Using the CYFRA 21-1 with the cut-off value of 5.3% ng/ml, the sensitivity was 58.6% and specificity was 95.9%. With the cut-off value of 3.3 ng/ml, sensitivity rose to 70.0%, without a marked loss of specificity (87.7%), and maintained the positive predictive value over 80%(Table 3).

Receiver operating characteristic (ROC) curves were generated from results of both tumor markers and areas under the curves (AUC) were calculated and compared. AUC of CYFRA 21-1(0.93) were significantly larger than that of SCC Ag (0.77) for the diagnosis of SQC ($p<0.05$)(Fig. 4).

DISCUSSION

Most of the current causes of increased cancer mortality are due to lung cancer, which is the most frequent fatal cancer in men and women, responsible for about 28% of all cancer deaths and about 6% of all deaths in the United Status[17]. Although the

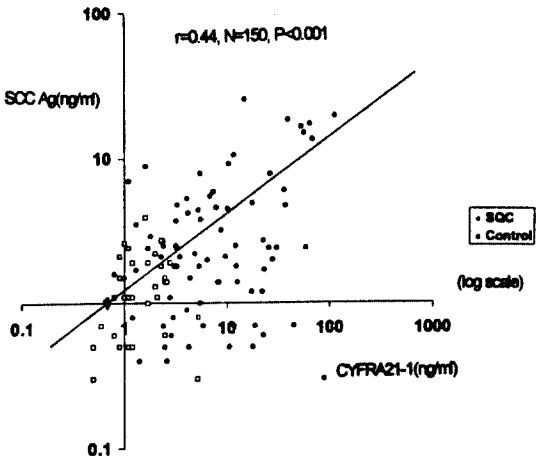

Fig. 3. Correlation between SCC Ag and CYFRA 21-1 in control subjects and patients with squamous cell lung cancer.

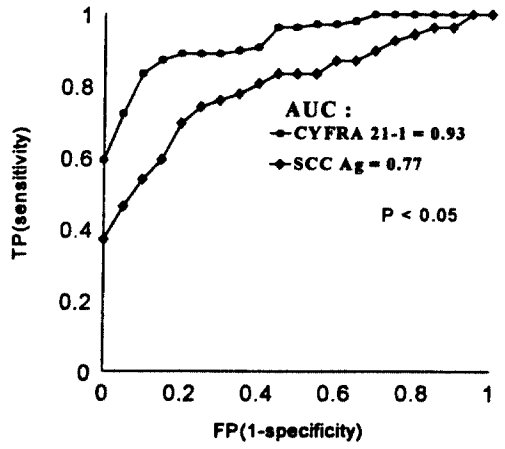

Fig. 4. Comparison of receiver operating characteristic curve of CYFRA 21-1 and SCC Ag for the diagnosis of SQC. FP : false positive, TP : true positive AUC : area under curve.

high rate of lung cancer is expected to plateau and begin to decline in the late 1990s in western countries, we are not so optimistic here because of increasing smoking rates and industrial or environmental pollution in Korea[18, 19].

The best chance to cure non-small cell lung carcinoma(NSCLC) is surgical resection but the proportion of surgical canditates, from all patients initially diagnosed, is only 20-25%. Therefore early detection in the resectable stage of disease would be a major impact on the survival of NSCLC patients. The value of screening procedures, however, was disappointing in a previous study[20].

Serum tumor markers have been studied for the basis of several clinical uses : as a screening for early diagnosis, as an aid to evaluate histologic types, as means for staging or defining the tumor volume and prognosis and lastly, for monitoring response to treatment and for detecting recurrence or metastasis[21]. In general, tumor markers can have value either as a screening test or as a test for disease monitoring for the known cancer. If a test has a low value in the early stage of disease, but increases according to advanced tumor stage, the test can be considered to be valuable for clinical follow-up. On the contrary, if a test reveals discriminating levels, regardless of tumor burden, the test would have a merit as a screenig tool.

Despite an increasing number of available tumor markers in carcinoma of the lung, utilization has been limited by insufficient sensitivity and specificity. Among them, carcinoembryonic antigen (CEA), squamous cell carcinoma associated antigen (SCC Ag) and, recently, cytokeratin fragment 19 have been reported to have value for lung cancer.

CEA, which is a glycoprotein of about 200,000 daltons, is present in large amounts in embryonic life and later in carcinoma of the colon and other malignancies[21]. In malignancies of the lung, serum levels of CEA are elevated in 68% of adenocarcinoma, 40% of squamous cell carcinoma, 49% of small cell undifferentiated carcinoma and 51% of large cell carcinoma[22]. However, as it is also found in smokers and in pateints with chronic bronchitis[23], the routine measurement of CEA for screening purpose is not recommended[24].

There have been studies[3-5] on the usefulness of serum SCC Ag in lung cancer. Mino et al.[3] found elevated SCC Ag in 59% of patients with squamous cell carcinoma of

the lung and in 18% of other cell types. In squamous cell carcinoma of the lung, the SCC Ag level became higher as the clinical stage advanced while, in other types of lung cancer, it remained low regardless of the clinical stage. Furthermore, they demonstrated that the increased level of serum SCC Ag decreased after radiation therapy. Upham et al.[4] reported that SCC Ag was specific for the squamous cell carcinoma of the lung and suggested the potential use of SCC Ag for the typing of histology in equivocal tissue findings. In view of the low sensitivity in the localized stage, SCC Ag has not been recommended as a screening test. However, it has been suggested that SCC Ag has a value in disease monitoring for squamous cell carcinoma as there is correlation between the tumor stage and the level of SCC Ag[3, 5]. Also it has a value in differential diagnosis, since a high concentration of this tumor marker is relatively specific[4, 5].

Serum cytokeratin fragment has been reported[7-9] as a useful diagnostic tool, especially for squamous cell carcinoma of the lung. In a multicenter study[7], with 2250 lung cancer patients and 771 healthy controls, CYFRA 21-1 showed 96% specificity with 3.3 ng/ml of cut-off value. Also, according to the tumor burden, significant increased in the advanced stage of disease was observed[8, 9]. Furthermore, Pujol et al.[9] demonstrated a significant negative prognostic effect of CYFRA 21-1 in squamous cell carcinoma of the lung. As this tumor marker showed correlation with the tumor burden, studies investigating the value for disease monitoring after therapy[10, 11], have been followed. In a study with a series of 76 patients with squamous cell lung cancer, Niklinski et al.[10] reported a value as an indicator for the early tumor recurrence after surgical resection. Furthermore, there is another study[11] suggesting a role as a marker for chemotherapeutic response.

In our result, the serum SCC Ag and CYFRA 21-1 were both significantly higher in lung cancer patients, compared with control subjects. However, as reported previously, the significant difference was noted only in squamous cell carcinoma. In squamous cell carcinoma, both markers showed higher serum levels according to the advanced stage of disease, which means that both tests have a role as indicators of tumor burden rather than as screening tests. However, as mentioned in a previous study[12], both tests showed no significant difference between stage IIIa and IIIb disease so that both have no role in discriminating the resectability for the cases with stage III diseases.

In comparison with these tumor markers, Stieber et al.[12] showed that the diagnostic sensitivity of CYFRA 21-1 in lung cancer was superior to CEA, SCC Ag and NSE. However, their study subjects were comprised of all histologic types of lung cancers. In our result, using only squamous cell carcinoma for comparison, CYFRA 21-1 revealed more discriminative distribution than SCC Ag which showed considerable overlap between cancer and control subjects. Therefore, the diagnostic sensitivity of SCC Ag could not be raised without significant loss of specificity, while, with CYFRA 21-1, it could be raised with the specificity relatively unaffected. We could further confirm this difference by comparing the area under ROC curves, which demonstrated significant difference between the tumor markers.

This study has several limitations. Firstly, although Niklinski et al.[10] noted an elevated level of CYFRA 21-1 in 61% of stage I squamous cell lung carcinoma, high sensitivity with specificity for the early stage disease is generally not expected, reflecting the correlation of level of CYFRA 21-1 with the tumor stage. As we have not enough cases with early stage disease, further study would be required to conclude the value as a screening method. Secondly, this study lacks some clinical variables to be able to conclude the correlation with tumor burden and prognosis. It would be advisable to collect autopsy or surgical data on tumor weight or volume, post-operative follow-up data as well as prognostic information.

For future study, there are several areas of

potential concern with CYFRA 21-1. Firstly, although we did not present the data from cavitary lesions, we have experienced several cases with increased serum cytokeratin level, not only in SQC but also in benign cavitary lung lesions. Considering the cell lysis or tumor necrosis as a mechanism for elevated serum cytokeratins, it would be required to study further benign and malignant lesions with cavity or necrosis. Secondly, for the differential diagnosis of exudative pleural effusions, CYFRA 21-1 may have a value in differentiating malignant effusions with those from inflammatory origin. We are currently investigating more cases to discover these points.

In conclusion, for the diagnosis of squamous cell carcinoma of the lung, CYFRA 21-1 and SCC Ag both are considered as useful diagnostic tools. The correlation of both tests with tumor burden suggest the possible roles for disease monitoring after therapy and as indicators of therapeutic response. In a comparison of both tumor markers, CYFRA 21-1 is superior to the SCC Ag in sensitivity and specificity for the diagnosis of squamous cell carcinoma of the lung.

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