Utility of Serum Cytokeratin 19 Fragment (CYFRA 21-1) and Carcinoembryonic Antigen (CEA) as Tumour Markers for Non-Small Cell Lung Cancer

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Abstract

Serum cytokeratin 19 fragment (CYFRA 21-1) and carcinoembryonic antigen (CEA) levels were determined with an enzyme immunoassay in 51 patients with non-small cell lung cancer (NSCLC), 26 patients with benign lung diseases and 26 normal individuals in order to evaluate their clinical utility in the diagnosis of NSCLC. Patients with NSCLC demonstrated higher serum CYFRA 21-1 and CEA levels than both patients with benign lung diseases and normal group. We used the cut off value which was derived from the 95 th percentile value of CYFRA 21-1 and CEA levels in the group of patients with benign lung diseases ; CYFRA 21-1 at 3.13 ng/ml and CEA at 7.7 ng/ml. The sensitivity and diagnostic accuracy of CYFRA 21-1 and CEA for the group of NSCLC patients were 66.7 per cent, 76.6 per cent and 35.3 per cent, 55.8 per cent, respectively. When combining CYFRA 21-1 with CEA, the sensitivity and diagnostic accuracy were 68.6 per cent and 66 per cent. These results suggest that CYFRA 21-1 and CEA are useful serum markers for the diagnosis of NSCLC ; especially subtype squamous cell and adenocarcinoma, respectively. The usefulness is not enhanced by combining the assay of CYFRA 21-1 and CEA.

Key word : CYFRA 21-1, CEA, Tumour Marker, Diagnosis, Non-small Cell Lung Cancer

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Lung cancer is an important health problem. It is now the most common cause of cancer death in both men and women[1]. Non-small cell lung cancer (NSCLC) is the major form and it remains a challenge because of its high incidence and poor prognosis. Local spread or distant metastasis at the time of diagnosis are very common[2-4]. Clinical history and a number of investigations

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including chest X-ray, CT chest scan, bronchoscopy and biopsy, transthoracic needle aspiration are used for the diagnosis. Despite these procedures, the diagnosis often remains elusive. Serum tumour markers have proved useful for identifying patients presenting with a mass lesion on the chest X-ray, who despite negative initial workup, need further diagnostic intervention[5].

Recently, there has been growing evidence of the usefulness of CYFRA 21-1, a fragment of cytokeratin subunit 19, in the management of NSCLC[6]. This new tumour marker has been shown to be sensitive and specific for squamous cell cancer[7]. The aims of this study were to determine serum levels of CYFRA 21-1 a new marker, and carcinoembryonic antigen (CEA) the most widely used tumour marker in NSCLC and to assess the individual and combined utility of the two tumour markers for NSCLC diagnosis.

MATERIAL AND METHOD

Subjects

Serum samples were taken pretherapeutically from 77 patients with lung cancer and benign lung diseases attending the Central Chest Hospital from June to August 1997. Of these patients as shown in Table 1, 51 had histologically confirmed non-small cell lung cancer (35 males, 16 females ; age ranged from 34 to 86 years with a median of 62 years). The histological examination was performed according to the World Health Organization and staging was classified according to the international staging system[8,9]. There were 13 squamous cell carcinomas, 37 adenocarcinomas and 1 large cell carcinoma. Among these, 9 were in stage I, 5 in stage II, 5 in stage III a, 25 in stage III b, and 7 in stage IV. The benign lung diseases group consisted of 26 patients (13 males, 13 females ; age ranged from 21 to 76 years with a median of 59 years). This group comprised 7 chronic obstructive pulmonary disease (COPD), 6 tuberculosis, 2 pneumonia, 7 cryptogenic fibrosing alveolitis (CFA), and 4 bronchiectasis.

The normal group consisted of 26 staff members. They had normal routine blood biochemical testing and no clinical evidence of cancer. There were 18 males and 8 females aged from 29 to 73 years with a median of 53 years.

Assay procedure

Serum CYFRA 21-1 and CEA levels were measured by an enzyme immunoassay using commercial kits of Boehringer Mannheim, Mannheim, Germany. The principles of the tests were based on a two-step and a one-step sandwich assay for CYFRA 21-1 and CEA, respectively. Using the streptavidin-biotin technology, the tests were performed at 25°C on the automated ES 22 analyzer. The control sera for tumour markers were also included.

Statistical analysis

Normal distribution was not assumed for the distribution of CYFRA 21-1 and CEA. Consequently, non-parametric statistics were used. The data of the markers were expressed in each set of the study group as median and range. Differences between the two independent groups were determined by median test. P-value less than 0.05 was considered as significant. The sensitivity and diagnostic accuracy of CYFRA 21-1 and CEA as serologic marker were established. Sensitivity was defined by the percentage of increased levels exceeding the 95 th percentile range of the reference group of patients suffering from benign lung diseases. The diagnostic accuracy was defined as the number of true positive (NSCLC) and true negative (benign lung diseases) divided by the total number of observations[10].

RESULTS

Normal group and benign lung disease group

In the 26 normal group, the serum CYFRA 21-1 and CEA levels ranged from 0.16 to 3.6 and 0.55 to 6.62 ng/ml with a median of 1.91 and 2.55 ng/ml, respectively. In the group of benign lung diseases, the median levels of serum CYFRA 21-1 and CEA were 1.36 ng/ml (range, 0.13 to 4.32) and 2.41 ng/ml (range, 0.8 to 8.6), respectively. There was no significant difference of these markers between the two groups.

Lung cancer group

In 51 NSCLC patients, the median levels of serum CYFRA 21-1 and CEA were 4.35 ng/ml (range, 0.33 to 54.9) and 6.4 ng/ml (range, 0.51 to 80.0), respectively. These median levels of both serum markers were significantly higher than those of the two control groups as shown in Table 2.

Diagnostic specificity

In order to compare our results for the tumour markers under the same conditions, we followed the recommendations of the "Hamburger

Table 1. Patient characteristics.

	NSCLC		Benign lung diseases
No. of patients	55		26
Male / Female	35/16		13/13
Median age (range), years	62 (34-86)		59 (21-76)
Histology			
		COPD	7
Squamous cell carcinoma	13	Tuberculosis	6
Adenocarcinoma	37	Pneumonia	2
Large cell carcinoma	1	Fibrosing alveolitis	7
		Bronchiectasis	4
Stage I	9		
II	5		
IIIa	5		
IIIb	25		
IV	7		

Table 2. Serum levels of CYFRA 21-1 and CEA in study groups. The data are expressed as median and range.

Study groups	n	CYFRA 21-1 (ng/ml)	CEA (ng/ml)
Lung cancers	51	4.35 (0.33 – 54.9)*	6.40 (0.51 – 80)*
Benign lung diseases	26	1.36 (0.13 – 4.32)	2.41 (0.8 – 8.6)
Normal individuals	26	1.91 (0.16 – 3.6)	2.55 (0.55 – 6.62)

* compared with benign lung diseases and normal individuals group at p-value < 0.005

Table 3. Cut off values of CYFRA 21-1 and CEA of the study group.

Study group	CYFRA 21-1 (ng/ml)	CEA (ng/ml)
Benign lung diseases (n = 26)	3.13	7.70
Normal individuals (n = 26)	3.34	6.57

Group for the Standardisation of Tumour Markers" and used the cut off value which was derived from the 95 th percentile value of CYFRA 21-1 and CEA in each study group. This basic postulation resulted in different cut off values (Table 3). For the normal group, the cut off value was 3.34 ng/ml for CYFRA 21-1 and 6.57 ng/ml for CEA. In patients with benign lung diseases, the cut off value changed to a small extent for CYFRA 21-1 (3.13 ng/ml) and to a greater extent for CEA (7.7 ng/ml).

Diagnostic sensitivity and accuracy

To examine the clinical utility of CYFRA 21-1 and CEA, we compared the true positive test results based on the control group, they were the patients with a variety of benign lung diseases which had their cut off levels of CYFRA 21-1 at 3.13 ng/ml and of CEA at 7.7 ng/ml.

The distributions of the CYFRA 21-1 and CEA concentrations at the above mentioned cut off levels are shown in Fig. 1 and Fig. 2.

The sensitivity and accuracy of CYFRA 21-1 and CEA in the diagnosis of NSCLC were 66.7 per cent, 76.6 per cent and 35.3 per cent, 55.8 per cent, respectively. The combined sensitivity of CYFRA 21-1 and CEA and accuracy were 68.60 per cent and 66.23 per cent as shown in Table 4.

Fig. 3 represents the sensitivity of CYFRA 21-1 and CEA in the diagnosis of NSCLC on the basis of histological cell type (a) and tumour stage (b). The sensitivities of CYFRA 21-1 and CEA in

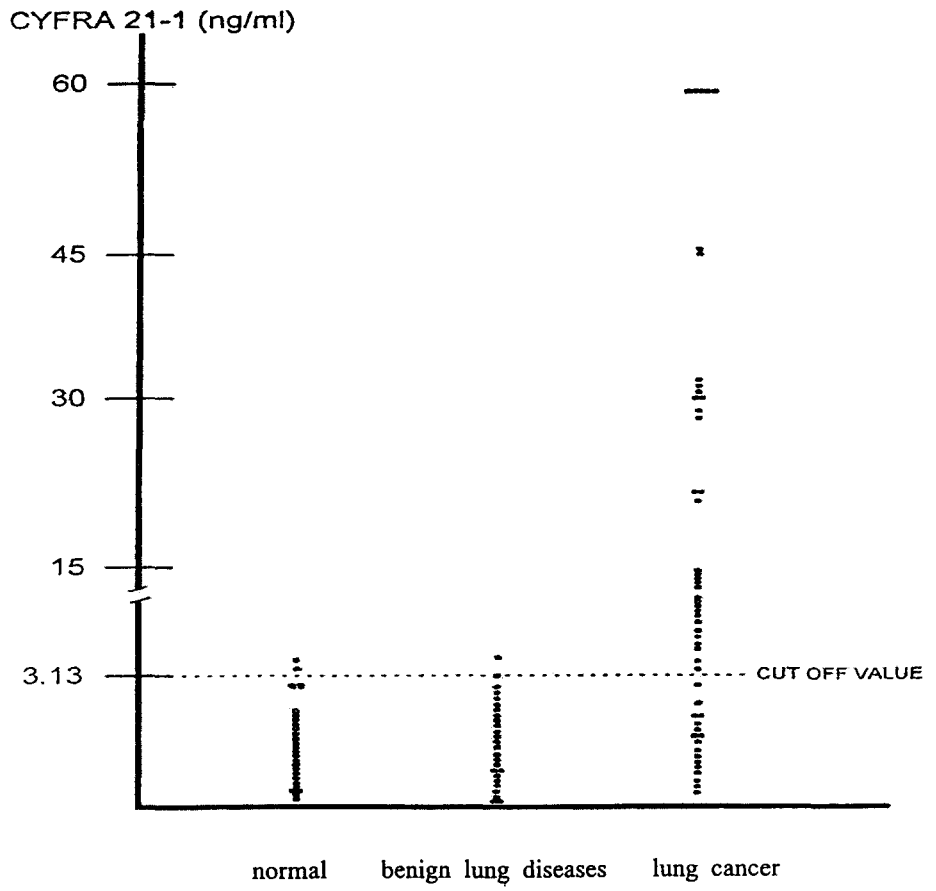

Fig. 1. Scattogram of serum CYFRA 21-1 value in normal individuals, patients with benign lung diseases, and non-small cell lung cancer. The dashed line indicates the cut-off value of CYFRA 21-1 at 3.13 ng/ml.

the diagnosis of squamous cell carcinoma were 69 per cent and 23 per cent, respectively; whereas, the sensitivities of CYFRA 21-1 and CEA in the diagnosis of adenocarciroma were 62 per cent and 43 per cent, respectively.

The sensitivities of CYFRA 21-1 and CEA in the diagnosis of NSCLC patients with stage I, II, IIIa, IIIb, and IV were 55, 80, 40, 76, and 43 per cent and 22, 40, 20, 40, and 57 per cent, respectively.

DISCUSSION

Cytokeratins are a family of cytoskeletal proteins with a molecular weight ranging from 40000 to 68000[11]. Cytokeratin 19 is expressed in normal epithelial cells and overexpressed in tumours of epithelial origin including all cell forms of the lung. CYFRA 21 – 1 is a fragment of cytokeratin 19 that may be released into the serum as cell lysis or tumour necrosis and may be a useful circulating tumour marker[12]. The profile assays of CYFRA 21-1 with other serum tumour markers are used for diagnosis of lung cancer[13,15]. In our study, the serum levels of CYFRA 21 – 1 and CEA were analysed in 51 NSCLC patients. The results showed that the median levels of both serum markers in NSCLC were significantly higher than those of the benign lung diseases group and normal individuals. The main results are in line with previous studies [16,17]. However, several issues arising from our study merit discussion.

Although the sensitivity, specificity, and accuracy are indicators of the usefulness of a diag-

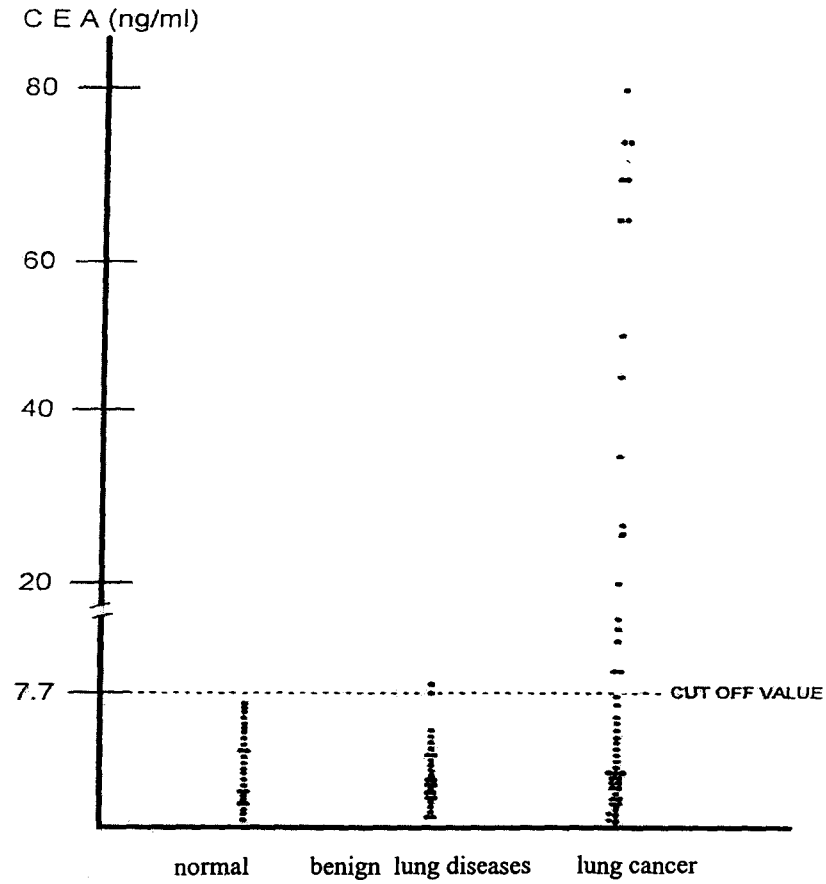

Fig. 2. Scattogram of serum CEA value in normal individuals, patients with benign lung diseases, and non-small cell lung cancer. The dashed line indicates the cut-off value of CEA at 7.7 ng/ml.

Table 4. Sensitivity and accuracy of CYFRA 21-1, CEA, and CYFRA 21-1 and CEA in the diagnosis of non-small cell lung cancer.

Markers	Sensitivity		Accuracy	
	Elevated/Total	%	Elevated/Total	%
CYFRA 21-1	34/51	66.66	59/77	76.62
CEA	18/51	35.29	43/77	55.84
CYFRA 21-1 and/or CEA	35/51	68.60	51/77	66.23

nostic test, a variety of criteria have been used to assess the diagnostic value of tumour markers. Initially, a fixed specificity to choose the cut off point has been recommended but the precise value used has varied between groups. A specificity of 100 per cent, although desirable in clinical terms, may not be practical because of the poor sensitivity that may follow. This is the reason why some groups have proposed using a specificity of 95 per cent as the cut-off point[15-17]. Nevertheless, the alternative

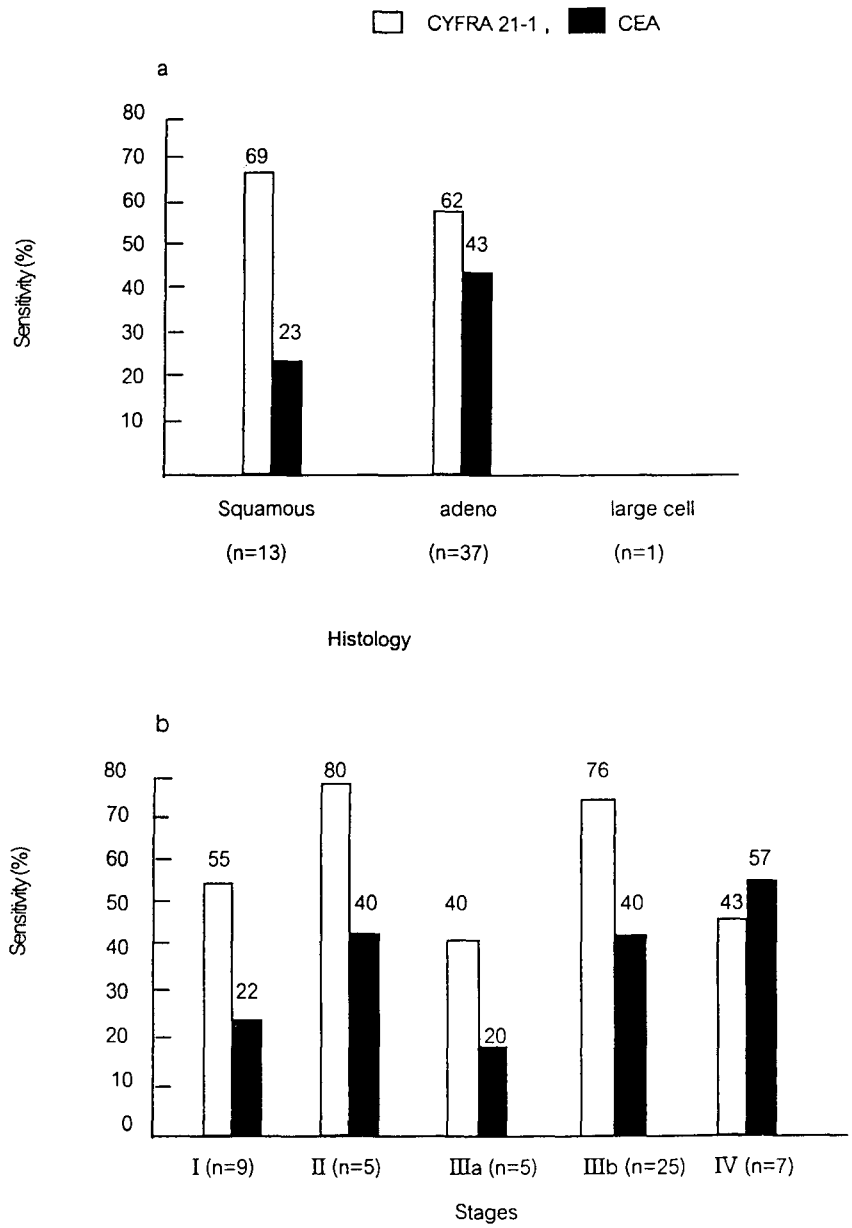

Fig. 3. Sensitivities of CYFRA 21-1 and CEA in diagnosis of non-small cell lung cancer on the basis of histological cell type (a) and tumour stage (b).

use of receiver operating characteristic (ROC) curves helps to preserve a higher sensitivity than when using the 100 per cent specificity as point of reference and in many cases avoid the burden of 5 per cent false positive cases[19,20]. In our study, we used a specificity of 95 per cent as the cut-off point following the recommendations of the Hamberger Group for the standardisation of tumour markers[21]. At the cut off level of 3.13 ng/ml for CYFRA 21-1 and 7.7 ng/ml for CEA, the sensitivities and accuracies of CYFRA 21-1 and CEA in detecting NSCLC are 66.7 per cent, 76.6 per cent and 35.3 per cent, 55.8 per cent, respectively. The combined sensitivity and diagnostic accuracy of

both markers are 68.6 per cent and 66.23 per cent, respectively. The cut off levels of CYFRA 21-1 and CEA from our results are very close to those of Ebert et al[18]. They used the cut off level at 3.3 ng/ml for CYFRA 21-1 and 7.8 ng/ml for CEA which was derived from 95 per cent specificity of 177 benign lung diseases, but reported lower sensitivities (50% for CYFRA 21-1 and 33% for CEA) and higher accuracies (84.1% for CYFRA 21-1 and 76.3% for CEA). In addition, their combined sensitivity of both markers in the detection of NSCLC (n=177) is 63 per cent. In contrast, Takada et al used the cut off level of CYFRA 21-1 at 3.5 ng/ml, established by the Japan CYFRA research group[16]. This cut off level was based on calculations using ROC curve instead of using the 95 per cent specificity. They found the sensitivity of CYFRA 21-1 in detecting NSCLC to be 70.5 per cent. In addition, they also compared the sensitivity of CYFRA 21-1 when using the cut off level at 95 per cent specificity. By using the cut off level at 5.5 ng/ml for CYFRA 21-1 and 4.2 ng/ml for CEA, the sensitivities of CYFRA 21-1 and CEA in detecting NSCLC are 49.5 per cent and 37.5 per cent, respectively.

It is obvious that the composition of the reference group influences the test results. Among the 26 patients with benign lung diseases from our study, elevated CYFRA 21-1 levels were found in only one case of COPD. Most of them had CYFRA 21-1 levels within the normal range, whereas, the CEA levels were clearly higher due to the well – known effect of smoking. By using a reference group heterogeneous of aetiology, clinical manifestation, and consideration of the few false – positive results, it is reasonable to state that a larger number of reference group provides a more reliable cut off level. Therefore, the differences of the sensitivities and diagnostic accuracies of the tumour markers in the detection of NSCLC from many investigators depend on choosing the cut off level from patients with benign lung diseases and the composition of the NSCLC patients.

Regarding the association of these markers with tumour histotype, the higher sensitivity of CYFRA 21-1 has been found in patients with squamous cell carcinoma than in patients with adenocarcinoma, whereas, the reverse is true for CEA. These results agree with the others[18,22]. The sensitivities of CYFRA 21-1 and CEA in patients with large cell carcinoma are not assessed because of the low number of cases in our sutdy. However, Ebert et al have reported that combined application of CYFRA 21-1 and CEA can increase the sensitivity for all types of lung cancer, especially for adenocarcinoma[18].

The relation to tumour stage, CYFRA 21-1 and CEA do not show a steady increase in sensitivity levels with tumour progression. The highest sensitivities of CYFRA 21-1 and CEA are found in patients with stage II and stage IV, respectively. In contrast, Stieber et al have found that the sensitivities of the two markers correlate with tumour stage[15]. Moreover, Bates et al have also reported that measurement of CYERA 21-1 and CEA at bronchoscopy can significantly increase that diagnostic yield in patients suspected of having lung cancer and is especially useful in those patients in whom tumour biopsy is not feasible at bronchoscopy[22]. A high level of CYFRA 21-1 in apparently early – stage NSCLC should be an indicator for more extensive workup before thoracotomy and an elevated level of CYFRA 21-1 at any time during the disease course is a predictor of poor survival[14,23]. In addition, Moro et al have reported that NSCLC patients with both high serum CYFRA 21-1 and CEA levels have a shorter survival than those with normal serum levels[24].

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การใช้ซีรั่มไซโตเคอราติน 19 แฟลกเม้นท์ (CYFRA 21-1) และคาร์ซิโนเอ็มบริโอนิคแอนติเจน (CEA) เป็นสารบ่งชี้มะเร็งสำหรับมะเร็งปอดชนิด non-small cell

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จากการตรวจหาระดับ CYFRA21-1 และ CEA ในน้ำเหลืองผู้ป่วยมะเร็งปอดชนิด non-small cell 51 คน, ผู้ป่วยด้วยโรคปอดชนิดอื่นๆ 26 คน และคนปกติ 26 คน ด้วยวิธีเอ็นไซม์อิมมูโนแอสเสย์เพื่อที่จะประเมินการใช้สารบ่งชี้มะเร็งทั้งสองนี้ทางคลินิกในการวินิจฉัยโรคมะเร็งปอดชนิด non-small cell พบว่าผู้ป่วยมะเร็งปอดชนิด non-small cell มีระดับ CYFRA 21-1 และ CEA สูงกว่ากลุ่มของผู้ป่วยด้วยโรคปอดชนิดอื่น ๆ และกลุ่มคนปกติ เมื่อใช้ค่า cut-off ซึ่งได้จากระดับ CYFRA 21-1 และ CEA ในกลุ่มผู้ป่วยโรคปอดชนิดอื่น ๆ ตรงตำแหน่งเปอร์เซนต์ไทล์ที่ 95 โดยใช้ CYFRA 21-1 ที่ 3.13 ng/ml และ CEA ที่ 7.7 ng/ml ความไวและความถูกต้องในการวินิจฉัยโรคของ CYFRA 21-1 และ CEA เท่ากับ 66.7%, 76.6% และ 35.3%, 55.8% ตามลำดับ เมื่อประเมิน CYFRA 21-1 และ CEA ร่วมกันความไวและความถูกต้องในการวินิจฉัยโรคเท่ากับ 68.6% และ 66% ผลการศึกษานี้แสดงว่าการตรวจ CYFRA 21-1 และการตรวจ CEA สามารถใช้ประกอบการวินิจฉัยมะเร็งปอดชนิด non-small cell โดยเฉพาะกลุ่มย่อย squamous cell และ adenocarcinoma ตามลำดับได้ แต่เมื่อใช้การตรวจ CYFRA 21-1 ร่วมกับการตรวจ CEA ความสามารถในการใช้ช่วยวินิจฉัยไม่ได้เพิ่มขึ้น

คำสำคัญ : ไซฟรา 21-1, ซีอีเอ, สารบ่งชี้มะเร็ง, การวินิจฉัย, มะเร็งปอดชนิด non-small cell

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