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## ORIGINAL ARTICLE

# Evaluation of HE4 in the Diagnosis and Follow Up of Non-Small Cell Lung Cancers

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## SUMMARY

***Background:*** **The aim of our study was to evaluate the performance of HE4 in the diagnosis and follow up in patients with non-small cell lung cancer (NSCLC).**
***Methods:*** **Serum levels of HE4, CEA, and Cyfra 21-1 were analyzed in 146 patients suspected with NSCLC and 30 healthy subjects to evaluate their diagnostic performance. A one year follow up was performed in 61 patients confirmed with NSCLC after surgery eradication at the interval of 1 month, 3 months, 6 months, and 12 months.**
***Results:*** **Our results showed that the area under the receiver operating characteristics curve (AUC) of HE4 was 0.761, which was similar with CEA. The sensitivity and specificity of HE4 was 0.82 and 0.62, respectively, at the level of 75.0 pmol/L. The AUC of HE4 was 0.70, 0.81, and 0.90 at 1 month, 3 months, and 6 months after surgery, respectively, which was significantly higher than CEA and Cyfra 21-1.**
***Conclusions:*** **Our finding indicates that HE4 is a potential marker for the diagnosis and follow up of NSCLC patients, which is complementary with CEA and Cyfra 21-1 and accurate in predicting NSCLC recurrence in early stage.**
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**KEY WORDS**

human epididymis secretory protein 4, non-small cell lung cancer, receiver operating characteristics curve, serum marker

## INTRODUCTION

The identification of diagnostic bio-markers of non-small cell lung cancer (NSCLC) is an important goal in routine clinical practice [1]. These markers may help to screen potential NSCLC patients in a healthy cohort or to differentiate NSCLC patients from benign lung disease [2,3]. Several serum markers have been recommended for this purpose, including CEA, Cyfra 21-1, and SCC [4-6]. However, the utility of these markers in clinical practices is still being disputed for the diagnostic sensitivity or specificity [2,7,8].
Human epididymis secretory protein 4 (HE4) is a secreted glycosylated protein belonging to the WFDC (previously named WAP) family. WAP four-disulfide

core domain 2 (WFDC2), the gene encoding HE4, is located on chromosome 20, in a segment frequently amplified in many cancers (breast, ovarian, colon, pancreas, and lungs) [9,10]. Many publications have shown that the serum levels of HE4 and CA125 can be used for the early detection of ovarian cancer recurrence and to classify patients with a pelvic mass as at high or low risk of ovarian malignancy [11-13]. The role of HE4 in ovarian malignancy was widely studied and well recognized. However, HE4 is not ovarian cancer-specific. Indeed, WFDC2 is strongly expressed in normal human trachea and salivary glands and, to a lesser extent, in lung, prostate, pituitary gland, thyroid, and kidney [14]. Moderate to high levels have also been detected in lung adenocarcinoma and, occasionally, in breast, transitional cell, and pancreatic carcinomas [15,16]. In recent years, emerging studies have suggested that HE4 could be a potential diagnostic and prognostic marker in NSCLC [17-19].

The aim of our study was thus to evaluate and compare the performance of HE4 in the diagnosis and follow up with other serum markers in patients with NSCLC.

## MATERIALS AND METHODS

### Patients and controls

Serum samples from 146 consecutive patients suspected with NSCLC referred to the Jiangmen Central Hospital, between January 2013 and December 2014, were collected for this study. Thirty age and gender matched healthy subjects were recruited in this study. The following investigations were carried out in all subjects: physical examination, standard chest roentgenography, computed tomography (CT) scan of chest, upper abdomen, and brain, fiberoptic bronchoscopes, liver sonography and bone scintigraphy. Mediastinoscopy was performed to establish the node status in patients with non-metastatic NSCLC, but with evidence of mediastinal lymph node enlargement on the chest CT images. Written informed consent was obtained from all subjects, and this study was approved by our institutional review board.

### Tumor marker detection

Blood samples were collected prior to surgery in serum separator tubes and were centrifuged, aliquoted, and frozen within 4 hours. The samples were stored at -20°C. At the clinical laboratory department, HE4 and CEA were tested using the ARCHITECT HE4 and CEA assays (Abbott Diagnostics, Abbott Park, IL, USA) according to the manufacturer's instructions. Serum Cyfra 21-1 level was detected with a Cyfra 21-1 test kit (Roche Diagnostics Corp, China) using a Cobas e601 analyzer.

### Diagnosis of NSCLC

Tissue specimens were obtained from patients suspected with NSCLC and a complete histopathological evaluation was performed. All histological results were performed blinded to laboratory biomarker detection technicians, and laboratory testing was performed blinded to histological results. NSCLC cancers were classified according to the WHO histological classification; however, the last revision concerning the new taxonomy of adenocarcinomas was not taken into account and adenocarcinoma was considered as a generic sub-histologic type [20]. Staging was carried out according to the Union for International Cancer Control (UICC) tumor node metastasis (TNM) classification in use at the time of diagnosis [21] and the American Thoracic Society map of regional pulmonary nodes [22].

### Follow up of NSCLC patients

For patients confirmed with NSCLC and who had received surgery therapy, we performed a one year follow up at the interval of 1, 3, 6, and 12 months after surgery. During follow up, patients received the same investigation as their first visits.

### Statistical analysis

Statistical analysis was analyzed by MedCalc 12.7.0.0 (MedCalc Software, Mariakerke, Belgium) and SPSS 19.0 (SPSS, Brussels, Belgium). One-way analysis of variance was used to compare the concentrations of serum samples and the two-sample *t*-test was used to compare pairwise mean values between groups. Other variables were evaluated by the chi-square test, Fisher's exact test, and Mann-Whitney *U* test when appropriate. The receiver operating characteristics (ROC) curve was used to evaluate the diagnostic value, and the areas under the curve (AUC) were compared by Z scores. A two-tailed p-value of less than 0.05 was considered statistically significant.

## RESULTS

### Demographic data of all subjects

In total 146 patients suspected with NSCLC, 19 patients were excluded for the lack of histopathological data, and 2 patients were confirmed with small cell lung cancer. 115 patients were finally enrolled in this study. The characteristics of 115 patients and 30 healthy controls were listed in Table 1. 82 patients were diagnosed as NSCLC including 64 patients with adenocarcinoma, 17 patients with squamous cell carcinoma and one patient with adenosquamous carcinoma. 33 patients were confirmed with benign lung disease. Age, gender, and smoking status were similar in the three groups. Mean levels of HE4, CEA, and Cyfra 21-1 were 91.5 ± 47.1 pmol/L, 24 ± 75.1 μg/L, and 5.1 ± 4.98 ng/mL in NSCLC patients respectively, which were significantly higher than benign lung disease and healthy controls. However, the serum levels of HE4, CEA, and Cyfra 21-1 were similar between healthy controls and benign lung disease.

**Table 1. Characteristics of all subjects.**

| | | NSCLC | Benign lung disease | Healthy control | p-value |
|---|---|---|---|---|---|
| Gender | Male | 49 | 18 | 17 | 0.763 [a] |
| | Female | 33 | 15 | 13 | |
| Age, years | Median | 62 | 58 | 57 | 0.836 [b] |
| | Range | 40 - 82 | 36 - 83 | 40 - 80 | |
| Smoking | Yes | 42 (51.3%) | 16 (48.4%) | 13 (43.3%) | 0.786 [a] |
| | Never | 40 (48.7%) | 17 (51.6%) | 17 (56.7%) | |
| Stage | I | 31 | | | |
| | II | 7 | | | |
| | III | 29 | | | |
| | IV | 15 | | | |
| Histology | Adenocarcinoma | 64 | | | |
| | Squamous cell carcinoma | 17 | | | |
| | Adenosquamous carcinoma | 1 | | | |
| | Benign sarcoidosis | | 13 | | |
| | Pulmonary tuberculosis | | 8 | | |
| | Organizing pneumonia | | 10 | | |
| | Lymphadenitis | | 1 | | |
| | Hamartoma | | 1 | | |
| Serum markers | HE4 (pmol/L) | 91.5 ± 47.1 | 65.4 ± 24.4 | 61.9 ± 14.9 | < 0.01 [c] |
| | CEA (µg/L) | 24 ± 75.1 | 1.7 ± 1.4 | 1.17 ± 0.81 | < 0.01 [c] |
| | Cyfra 21-1 (ng/mL) | 5.1 ± 4.98 | 2.43 ± 1.44 | 2.56 ± 1.18 | 0.03 [c] |

[a] - analyzed by Chi Squared test, [b] - analyzed by Mann-Whitney U test, [c] - analyzed by One-way ANOVA.

**Table 2. The diagnostic value of three serum markers for NSCLC.**

| | AUC (95% CI) | Cutoff | Sensitivity | Specificity | NPV | PPV |
|---|---|---|---|---|---|---|
| HE4 (pmol/L) | 0.761 (0.659 - 0.822) | 75.000 | 0.622 | 0.820 | 77.880 | 67.860 |
| CEA (µg/L) | 0.812 (0.624 - 0.854) | 2.100 | 0.831 | 0.591 | 65.140 | 78.990 |
| Cyfra 21-1 (ng/mL) | 0.686 (0.601 - 0.772)[a] | 4.500 | 0.378 | 0.951 | 56.220 | 77.690 |

NPV - negative predict value, PPV - positive predict value, AUC - Area under curve. Comparison of AUC was analyzed by MedCal software using Delong's method.

### Diagnostic performance of HE4 in NSCLC

The diagnostic values of HE4, CEA, and Cyfra 21-1 were analyzed by ROC curve. The area under ROC curve (AUC) was 0.761, 0.81, and 0.686 for HE4, CEA, and Cyfra 21-1, respectively (Table 2 and Supplement Figure1). Comparison of AUC indicated that both HE4 and CEA were better than Cyfra 21-1 in the diagnosis of NSCLC. However, there was no significant difference between HE4 and CEA (Table 2). The cutoff value was defined as the maximum Youden index (sensitivity + specificity) in this study. Using this rule, the specificity and sensitivity was 0.82 and 0.62, respectively, for HE4. With the cutoff value of 2.1 µg/L, the sensitivity and specificity of CEA was 0.83 and 0.59, respectively. Cyfra 21-1 achieved a specificity of 0.951 at the cutoff value of 0.686 ng/mL; however, the sensitivity was 0.378.

Linear regression was performed to study the correlation of these three serum markers in NSCLC patients. No significant correlation was found between HE4,

**Table 3. Concentration of HE4, CEA, and Cyfra 21-1 in different stages of NSCLC patients.**

| | | Stage I (31) | Stage II (7) | Stage III (29) | Stage IV (15) | p-value |
|---|---|---|---|---|---|---|
| First measurement | HE4 (pmol/L) | 66.3 ± 21.5 | 71.6 ± 26.5 | 92.4 ± 24.26 [b] | 138.1 ± 78.3 [b] | < 0.01 [a] |
| | CEA (µg/L) | 2.55 ± 1.88 | 3.35 ± 1.93 | 40.1 ± 132.1 [b] | 101.3 ± 152.1 [b] | < 0.01 [a] |
| | Cyfra 21-1 (ng/mL) | 2.85 ± 1.76 | 3.72 ± 7.31 | 5.24 ± 3.53 [b] | 9.87 ± 8.15 [b] | < 0.01 [a] |
| Last Follow-up | | Stage I (26) | Stage II (5) | Stage III (10) | Stage IV (0) | p-value |
| | HE4 (pmol/L) | 65.7 ± 28.3 | 70.6 ± 23.7 | 84.5 ± 27.25 [b] | | < 0.01 [a] |
| | CEA (µg/L) | 2.95 ± 1.72 | 3.64 ± 1.73 | 20.2 ± 39.0 [b] | | < 0.01 [a] |
| | Cyfra 21-1 (ng/mL) | 2.59 ± 1.59 | 3.21 ± 5.36 | 5.014 ± 3.19 [b] | | < 0.01 [a] |

[a] - Difference in groups was analyzed by One-way ANOVA, [b] - Difference between stages was compared by two sample *t*-test. All three markers in Stage III and Stage IV were significantly higher than Stage I and Stage II $p < 0.01$).

**Table 4. Diagnostic performance of CEA, HE4, and Cyfra 21-1 in NSCLC recurrence.**

| Serum markers | Follow up interval | AUC (95% CI) | Sensitivity | Specificity | NPV | PPV | Cutoff |
|---|---|---|---|---|---|---|---|
| CEA | 1 month | 0.433 (0.235 - 0.632) | 0.501 | 0.423 | 0.881 | 0.082 | 2.160 |
| | 3 months | 0.741 (0.235 - 0.632) | 0.667 | 0.836 | 0.901 | 0.492 | 2.160 |
| | 6 months | 0.793 (0.465 - 0.891) | 0.830 | 0.700 | 0.900 | 0.542 | 2.160 |
| | 12 months | 0.921 (0.837 - 0.973) | 0.890 | 0.900 | 0.921 | 0.850 | 2.160 |
| HE4 | 1 month * | 0.704 (0.532 - 0.876) | 0.667 | 0.721 | 0.951 | 0.210 | 75.000 |
| | 3 months * | 0.814 (0.613 - 0.912) | 0.751 | 0.801 | 0.930 | 0.584 | 75.000 |
| | 6 months * | 0.905 (0.801 - 0.972) | 0.780 | 0.910 | 0.909 | 0.791 | 75.000 |
| | 12 months | 0.928 (0.821 - 0.969) | 0.900 | 0.940 | 0.930 | 0.900 | 75.000 |
| Cyfra 21-1 | 1 month | 0.537 (0.441 - 0.834) | 0.651 | 0.662 | 0.942 | 0.172 | 4.500 |
| | 3 months | 0.537 (0.321 - 0.714) | 0.580 | 0.560 | 0.841 | 0.241 | 4.500 |
| | 6 months | 0.742 (0.638 - 0.841) | 0.750 | 0.740 | 0.870 | 0.561 | 4.500 |
| | 12 months | 0.901 (0.798 - 0.943) | 0.830 | 0.950 | 0.891 | 0.911 | 4.500 |

NPV - negative predict value, PPV - positive predict value, AUC - area under curve. Comparison of AUC was analyzed by MedCal software using Delong's method. * - indicate that compared with other markers, $p < 0.05$.

CEA, and Cyfra 21-1 levels (relationship between HE4 and CEA, $r^2 = 0.23$; relationship between HE4 and Cyfra 21-1, $r^2 = 0.42$).

Multivariate regression was also performed to identify the role of CEA, HE4, and Cyfra 21-1 in the diagnosis of NSCLC. Our model showed a close relationship between these markers and the diagnosis of NSCLC ($r^2 = 0.341$, $p < 0.05$, Supplement Table 1).

We further analyzed the concentration of HE4, CEA, and Cyfra 21-1 in different disease stages (Table 3). Our data showed that the mean concentration of HE4, CEA, and Cyfra 21-1 varied in different disease stages. CEA and Cyfra 21-1 concentration in patients with stage III and stage IV were significantly higher than in patients with stage I and stage II.

**Evaluation of HE4 in the follow up of NSCLC patients**

In our study, a total of 61 patients received surgery eradication in our hospital, and 16 patients were lost during follow up, 5 patients died during this period, only 41 patients completed the one year follow up. NSCLC recurrence was determined using clinical assessment, radiographic reports, and/or data from biopsies. Recurrence within the lymph nodes was defined when a new or enlarging lymph node was more than 1 cm on the short axis on a follow-up CT scan. 12 patients were classified as recurrence, including 9 patients, 2 patients, and 1 patient from stage III, stage II, and stage I, respectively. 29 patients were classified as recurrence free. Serum concentrations of HE4, CEA, and

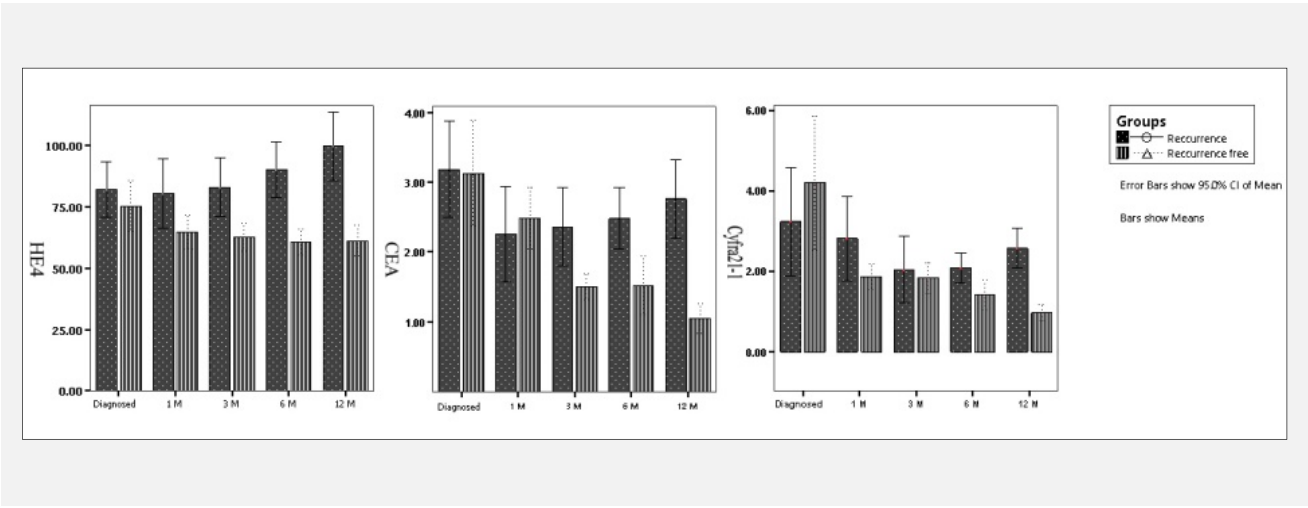


**Figure 1. Serum levels of HE4, CEA, and Cyfra 21-1 from follow up patients at different visit intervals.**

**As shown in the figure, in recurrence free patients, all three markers presented decreased continuously. While in recurrence patients, the trends in these serum markers were quite different.**

Cyfra 21-1 from these patients at different visit intervals were shown in Figure 1. In recurrence free patients, all three markers decreased within one month after surgery, and the decreasing of serum markers lasted up to 12 months, which was the end of our follow up. However, in patients with NSCLC recurrence, the trends in these serum markers were quite different. CEA and Cyfra 21-1 significantly decreased in the first month and 3 months after surgery, respectively, but HE4 showed little difference in 3 months after therapy (Figure 1). With disease progression, CEA and Cyfra 21-1 significantly increased at month 12 after therapy, but HE4 significantly increased at month 6. Based on these findings, we performed a ROC curve analysis to evaluate whether the concentration of these markers could correctly predict the recurrence of NSCLC at different intervals after surgery therapy (Table 4 and supplement Figure 2). The AUC of HE4 was 0.70, 0.81, and 0.90 at 1 month, 3 months, and 6 months, respectively, which was significantly higher than CEA and Cyfra 21-1. However, at 12 months after surgery, these markers showed no difference in distinguishing recurrence in patients. These findings were further supported by multivariate regression of combining the three markers (Supplement Table 1).

## DISCUSSION

HE4 is a cross-class protease inhibitor which is expressed in normal reproductive tracts and respiratory epithelium of the proximal airways. Although the increased expression of HE4 in tumor tissues and cell lines of lung cancer was described several years ago, in the past 2 years, researchers have begun studying the clinical significance of tumoral HE4 expression and the potential role of HE4 as a serum biomarker for the diagnosis of NSCLC.

Our study enrolled 115 patients suspected with NSCLC and 30 healthy controls, in which 82 patients were confirmed with NSCLC. We found that the HE4, CEA, and Cyfra 21-1 level at diagnosis in patients with NSCLC were significantly higher than patients with benign lung diseases or healthy subjects. The ROC curve, constructed using the sensitivity-specificity relationship, was used to determine the diagnostic value of these markers. The AUCs of three serum markers indicate that the diagnostic performance of HE4 and CEA were similar, but both were better than Cyfra 21-1. At the optimal cutoff point determined with the Youden's index, serum HE4 tended to present higher specificity, while CEA tended to present higher sensitivity. The relationship in HE4, CEA, and Cyfra 21-1 were further evaluated by linear regression and multivariate regression. Our results further identified the role of these three markers in the diagnosis of NSCLC and indicated that HE4 is independent and complementary with other serum markers in the diagnosis of NSCLC. However, our results showed a slight difference from other reports. Iwahori reported a ROC-AUC of 0.988 for HE4 in the diagnosis of NSCLC. The discrepancy could be explained by the different control populations used in the two studies (only healthy population in the work by Iwahori et al. study) [18]. Previous studies have indicated that HE4 is not tumor-specific and would increase in various disease conditions. Our diagnostic study encompassed both healthy individuals and patients with a variety of benign lung diseases as a control group, which may decrease the diagnostic specificity of HE4. However, our results were similar with the studies from Lamy and Chen

[7,17]. Levels of HE4, CEA, and Cyfra 21-1 were further analyzed to evaluate whether these markers reflect disease status. Our data showed that patients with stage III and stage IV showed higher levels of HE4, CEA, and Cyfra 21-1 than patients with stage I and stage II. These data showed that all these three markers can aid in NSCLC staging.

The value of HE4 in the follow up of NSCLC patients has not been well studied. We further analyzed the follow up value of HE4, CEA, and Cyfra 21-1 in 41 patients who had received surgery therapy in our hospital. These patients received strict investigation at 1 month, 3 months, 6 months, and 12 months after surgery to confirm their disease status. Serum level of HE4 presented a different pattern in recurrence patients compared with recurrence free patients. The serum HE4 concentration showed little change at 1 month and started to increase at 3 months after surgery. Using the cut-off value of 75 pmol/L, the sensitivity and specificity of HE4 in predicting NSCLC recurrence was 0.75 and 0.80, respectively at 3 months after surgery. At 6 months after surgery, the sensitivity and specificity was 0.78 and 0.91, respectively. Since most of the patients were confirmed with NSCLC recurrence at month 12 after surgery, HE4 could correctly predict NSCLC recurrence at an earlier stage, which showed better performance than CEA or Cyfra 21-1. However, our data showed that at month 12 after surgery, the diagnostic accuracy of HE4 was similar with CEA or Cyfra 21-1 in distinguishing recurrence and recurrence free patients. Although our results suggested that the specificity of HE4 was better than CEA and Cyfra 21-1 in the follow up of NSCLC patients, the level of HE4 may be affected by age, gender, and renal function. Attention should be paid to these factors when using our results.

Limitations of our study should be noted. First, the number of subjects enrolled in our study was somewhat smaller than other reports [17,18,23]. Secondly, the follow up period was only 12 months in our study, and whether HE4 plays an important role in the prognosis of NSCLC patients was not studied. Third, all subjects enrolled in our study were from the local urban area of Jiangmen City, the data may include some bias originating from this area or population. The lost rate was 26% in our study. The main reason for high lost rate was that many patients left and went to other hospitals during the follow up period. Taken together, our data deserve further verification in a larger population and multiple centers.

## CONCLUSION

HE4 is a potential marker for the diagnosis and follow up of NSCLC patients, which is complementary with CEA and Cyfra 21-1 and accurate in predicting NSCLC recurrence in early stage.

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**Declaration of Interest:**
The authors have declared that no competing interests exist.

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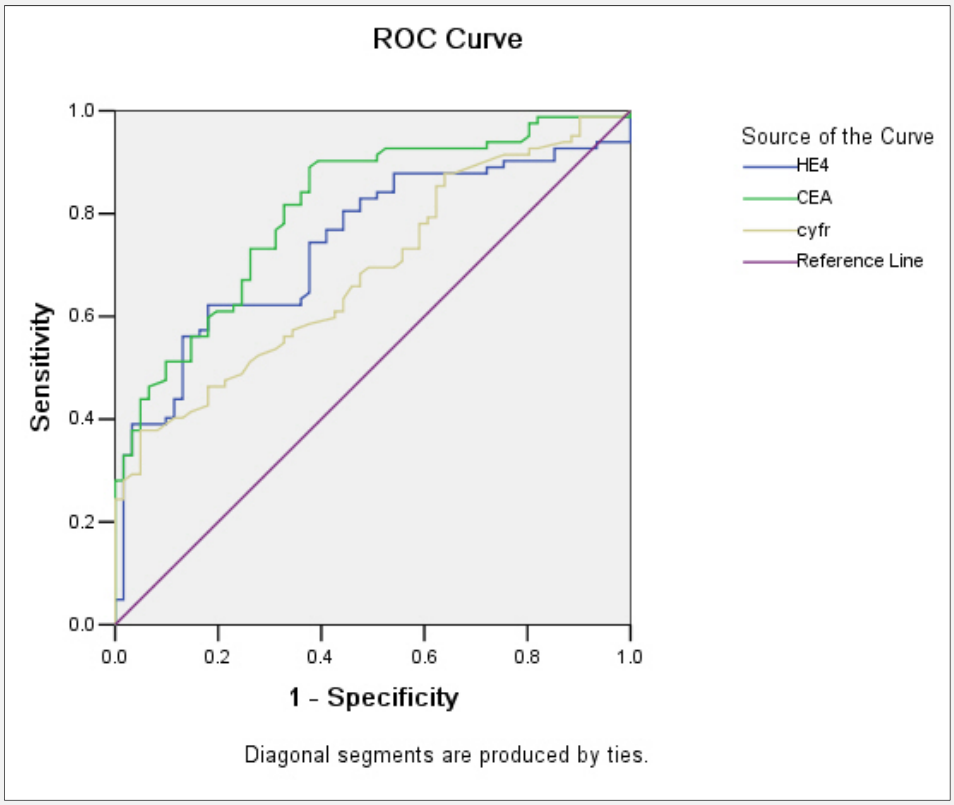
**Supplement Table 1. Multivariate regression of serum markers in predicting NSCLC**

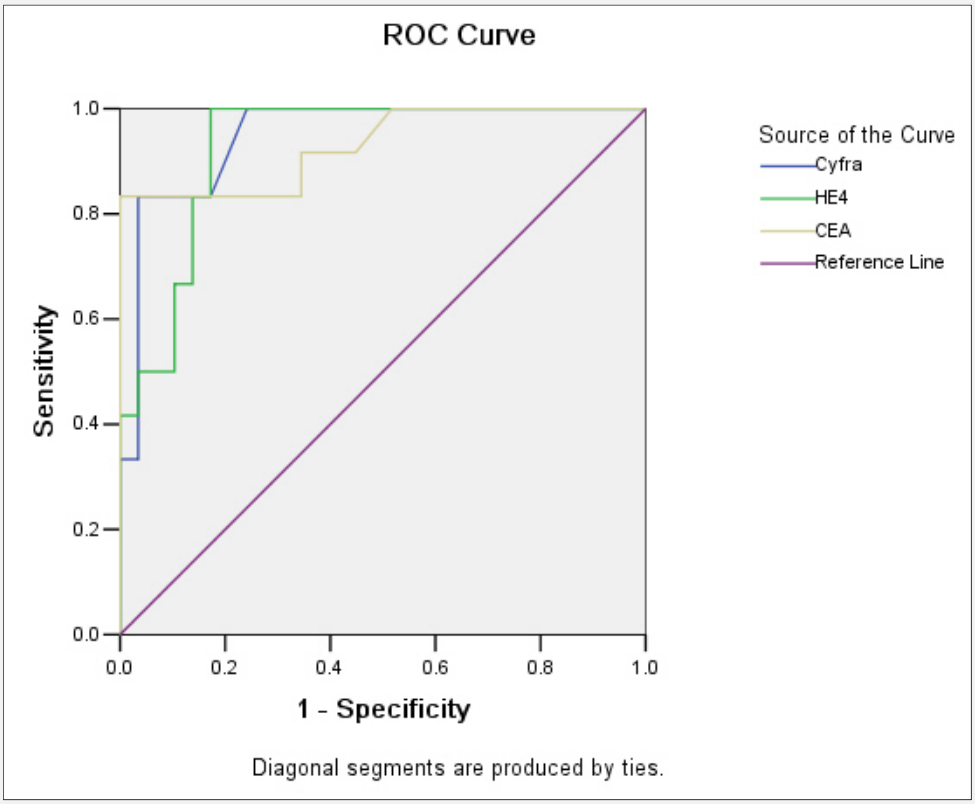
| | Equations | $r^2$ | p-value |
|---|---|---|---|
| First visit | $Y = 0.45 + 0.03X_1 + 0.05X_2 + 0.14X_3$ | 0.341 | 0.02 |
| 1 month | $Y = 0.76 + 0.07X_1 + 0.12X_2 + 0.36X_3$ | 0.126 | 0.12 |
| 3 months | $Y = 0.51 + 0.06X_1 + 0.11X_2 + 0.18X_3$ | 0.289 | 0.09 |
| 6 months | $Y = 0.40 + 0.05X_1 + 0.09X_2 + 0.26X_3$ | 0.261 | 0.02 |
| 12 months | $Y = 0.29 + 0.03X_1 + 0.24X_2 + 0.25X_3$ | 0.591 | < 0.01 |

**Multivariate linear regression was performed by entering the method. $X_1$ stands for HE4. $X_2$ stands for CEA. $X_3$ stands for Cyfra 21-1.**




**Supplement Figure 1. Diagnostic value of HE4, CEA, and Cyfra 21-1 in subjects.**

**As indicated in the figure, both CEA and HE4 presented higher AUC than Cyfra 21-1.**




**Supplement Figure 2. Predictive value of HE4, CEA, and Cyfra 21-1 at 12 months after surgery.**

**As indicated in the figure, all three markers presented good performance for discriminating recurrence and recurrence free patients at 12 months after surgery. No significant difference was found between these markers.**