Int J Clin Exp Med 2015;8(10):19014-19021
www.ijcem.com /ISSN:1940-5901/IJCEM0014373

Original Article

Value of serum marker HE4 in pulmonary carcinoma diagnosis

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Received August 12, 2015; Accepted October 10, 2015; Epub October 15, 2015; Published October 30, 2015

Abstract: An effective blood test is valuable to aid clinicians in making case management decisions. The present study was to analyze the value of four serum tumor markers for the diagnosis of pulmonary carcinoma. The case group consisted of 80 pulmonary carcinoma patients, which were compared to a control group of 30 patients with benign pulmonary disease and a control group of 30 healthy individuals. Serum levels of carcinoma embryonic antigen (CEA), cytokeratin protein fragment 21-1 (CYFRA21-1), neuron-specific enolase (NSE), and human epididymis protein 4 (HE4) were detected using electrochemiluminescence. Serum CEA, NSE, CYFRA21-1, and HE4 levels were significantly higher in pulmonary carcinoma patients than those in both control groups ($P < 0.05$). Serum CEA and HE4 levels were significantly higher in adenocarcinoma patients, while serum CYFRA21-1 levels were significantly higher in squamous cell carcinoma patients and serum NSE levels were significantly higher in small cell lung cancer (SCLC) patients ($P < 0.05$). Analysis of area-under-the-receiver operating characteristic (ROC) curves (AUC) revealed that serum CYFRA21-1, CEA, and HE4 levels were valuable for squamous cell carcinoma, serum CEA and HE4 levels were valuable for adenocarcinoma, and serum NSE level was valuable for SCLC ($P < 0.05$). Serum CEA and HE4 levels of pulmonary carcinoma patients with metastasis were higher than those with TNM stage I-II or III-IV disease without metastasis. In brief, detection of serum HE4 levels may be useful in auxiliary diagnosis and evaluation of the progression of pulmonary carcinoma.

Keywords: Carcinoma embryonic antigen, cytokeratin protein fragment 21-1, neuron specific enolase, human epididymis 4, serum markers, pulmonary carcinoma

Introduction

Pulmonary carcinoma is a malignant cancer with relatively high global incidence and fatality rates. The annual incidence of new pulmonary carcinoma cases is about 1.6 million worldwide, comprising 13% of all malignant tumor cases. The annual mortality of pulmonary carcinoma is about 1.4 million people worldwide, accounting for 18% of deaths attributed to malignant tumors [1]. According to the World Health Organization, the number of pulmonary carcinoma deaths in September 2011 exceeded total deaths by mammary adenocarcinoma, prostatic carcinoma, and colorectal carcinoma [1]. Statistical data released by the China Cancer Center indicates that China's annual incidence of new pulmonary carcinoma cases is about 0.6 million, and the country's mortality is about 0.2 million. Pulmonary carcinoma has become a serious public health tissue [2, 3].

Pulmonary carcinomas are mainly classified into three pathological types: non-small-cell lung cancer (NSCLC), small-cell lung cancer (SCLC), and undifferentiated carcinoma. About 80% of pulmonary carcinomas are NSCLC, which includes predominantly squamous cell lung carcinoma and pulmonary adenocarcinoma [4].

Statistical research indicates that the prognosis of pulmonary carcinoma is closely associated with clinical staging at diagnosis: postoperative five-year survival rate of patients at stage 0 is > 90%; postoperative five-year survival rate of patients at stage Ia is > 60%; and five-year survival rate of patients at stage II-IV is < 5%-40% [2, 3]. However, pulmonary carcinomas present no specific early-stage symptoms, so there are no clinical measures to screen and diagnose early disease. Therefore, there is a relatively low early diagnosis rate. About 60% of

pulmonary carcinoma patients are diagnosed at advanced stage and thus miss the best time for treatment [5]. Although platinum-containing two-medicine combinations and targeted therapy regimens somewhat improve treatment efficacy of advanced pulmonary carcinoma patients [6-8], overall survival rates remain unchanged and overall prognosis remains relatively poor [9].

Therefore, better means of early diagnosis is important for improving prognosis of patients with pulmonary carcinoma. Histopathological biopsy by bronchoscope, mediastinoscope, or thoracentesis is the most reliable method to diagnose NSCLC, but such invasive examinations cannot serve as main measures of early screening or continuous monitoring. Common early diagnosis methods include imaging, endoscopy, and molecular biology technologies, in addition to traditional methods such as chest X-ray, Nuclear Magnetic Resonance Imaging (MRI), Position emission tomography-Computer tomography (PET-CT), High Resolution CT (HRCT), low-dose CT (LDCT), autofluorescence bronchoscopy (AFB), endobronchial ultrasonography (EBUS), Fibered Confocal Fluorescence Microscopy (FCFM), Elemental carbon (EC), Electromagnetic Navigation (ENB), Transbronchial Needle Aspiration (TBNA), exhaled air analysis, and serum tumor marker joint detection-have been gradually applied to clinical studies.

Serum marker detection has various advantages, such as technical facility, low detection price, noninvasiveness, easy access to samples, and continuous monitoring; therefore, it is a high-profile topic in auxiliary diagnosis of early pulmonary carcinoma [10]. Current serum tumor markers have expanded the previous range, with over 200 kinds of tumor antigens, hormones, enzymes, isozymes, oncogenes, anti-oncogenes, and corresponding products now available. Markers' roles have expanded from pure tumor diagnosis to the prediction of tumor recurrence and metastasis, assessment of efficacy and prognosis, and mass survey.

Clinical studies have successively analyzed and explored a variety of tumor markers, such as carcino-embryonic antigen (CEA), cytokeratin fragment 21 (CYFRA21-1), neuron specific enolase (NSE), carbohydrate antigen (CA-199), cytokeratin 5/6 (CK 5/6), cytokeratin HMW (CK-HWM), thyroid transcription factor-1 (TTF-1), and cytokeratin 8/18 (CK 8/18), but no reliable serum indicators are independently applicable for early diagnosis of pulmonary carcinoma [11]. Investigators currently improve the efficiency of serum markers in early diagnosis of pulmonary carcinoma mainly by means of joint detection [12].

Human epididymis protein 4 (HE4) is a recently identified serum tumor marker. Studies have verified high expression in multiple tumors, making HE4 a potential marker for early diagnosis and recurrence monitoring of malignant tumors, such as ovarian cancer, endometrial carcinoma, and mammary adenocarcinoma [13-16]. In addition, HE4 is closely associated with occurrence, development, and prognosis of pulmonary carcinoma [17]. Therefore, this study further analyzed the value of HE4 compared to conventional serum markers for diagnosis of pulmonary carcinoma.

Methods

Study population

The case group included 80 patients with pulmonary carcinoma admitted into Affiliated Yancheng Hospital, School of Medicine, Southeast University (Yancheng, China) from January-April 2015. All patients were verified to have pulmonary carcinoma by imaging examination, such as CT and MRI, and the diagnosis was confirmed by pulmonary tissue biopsy or postoperative pathological examination. The group included 55 men and 25 women, 49-84 years old with a mean age of 65.2±9.8 years. Regarding pathological types, 29 patients had squamous carcinoma, 21 had adenocarcinoma, 16 had SCLC, and 14 had undifferentiated carcinoma.

The benign control group included 30 patients with benign pulmonary disease that visited our hospital during the same period. The group included 21 men and 9 women, aged 44-81 years old with a mean age of 63.8±8.7 years. Twenty-five patients had pneumonia and 5 had tuberculosis.

The healthy control group included 30 individuals who were healthy as verified by physical examination in Affiliated Yancheng Hospi-

Table 1. Comparison of serum marker levels

Groups	n	CEA (ng/mL)	NSE (ng/mL)	CYFRA21-1 (ng/mL)	HE4 (pmol/L)
Case group	80	46.09±39.56[b,c]	22.57±33.76[b,c]	40.86±44.35[b,c]	175.18±167.22[b,c]
Benign control	30	14.52±3.06[a]	11.26±6.79[a]	8.07±3.91[a]	34.04±4.77[a]
Healthy control	30	14.43±2.92[a]	8.75±5.23[a]	7.52±4.03[a]	29.67±5.37[a]
F		18.901	21.122	16.428	21.883
P		< 0.001	< 0.001	< 0.001	< 0.001

[a]$P < 0.05$, *vs* case group; [b]$P < 0.05$, *vs* benign control group; [c]$P < 0.05$, *vs* healthy control group.

Table 2. Comparison of serum marker levels among pulmonary carcinoma pathologies

Group	n	CEA (ng/ml)	NSE (ng/mL)	CYFRA21-1 (ng/mL)	HE4 (pmol/L)
Squamous cell carcinoma	29	36.36±25.24[b]	17.93±6.73[c]	93.86±29.85[b,c,d]	125.05±89.06[b]
Adenocarcinoma	21	94.18±41.31[a,c,d]	18.23±7.64[c]	13.06±9.70[a]	357.04±220.40[a,c,d]
Small cell lung cancer	16	22.73±9.91[b]	41.28±19.17[a,b,d]	9.23±4.71[a]	101.51±44.23[b]
Undifferentiated carcinoma	14	20.79±9.62[b]	17.30±7.69[c]	8.89±3.65[a]	90.41±49.04[b]
F		31.981	20.361	118.612	19.287
P		< 0.001	< 0.001	< 0.001	< 0.001

[a]$P < 0.05$, *vs* squamous cell carcinoma; [b]$P < 0.05$, *vs* adenocarcinoma; [c]$P < 0.05$, *vs* small cell lung cancer; [d]$P < 0.05$, *vs* undifferentiated carcinoma.

tal, School of Medicine, Southeast University (Yancheng, China) during the same period. The group included 20 men and 10 women, 41-79 years old with a mean age of 62.2±8.6 years. Clinical examination ruled out lesions in all major vital organs.

Differences among the three groups in terms of age and gender composition were not statistically significant ($P > 0.05$). All study subjects provided written informed consent. This study protocol was approved by the Medical Ethic Committee of Affiliated Yancheng Hospital, School of Medicine, Southeast University.

Serum measurements

Morning fasting peripheral blood samples were collected from study subjects of the three groups. For patients in case and benign control groups, collection time was before the patients received relevant treatment. Samples were retained for 1 hour at room temperature, then serum was isolated by centrifugation at 4000 rpm. Isolated serum was stored at -20°C.

The levels of HE4, CEA, NSE, and CYFRA21-1 were detected in serum samples by Electrochemiluminescence. The matched reagents and purchased from Roche (Basel, Switzerland) and used as directed. All detection was conducted according to protocols.

Statistical methods

SPSS 13.0 statistical package was used to establish a database and perform statistical analyses. Measurement data were expressed as mean ± standard deviation. Single factor analysis of variance was adopted for intergroup comparison, and LSD method was utilized for pairwise comparison. Area-under-the-receiver operating characteristic (ROC) curve (AUC) was used to compare serum marker diagnostic efficiency. $P < 0.05$ was considered statistically significant.

Results

Comparison of serum marker levels

Serum levels of CEA, NSE, CYFRA21-1, and HE4 significantly differed among case, benign control, and healthy control groups (F = 18.901, 21.122, 16.428, 21.883, respectively). Serum marker levels were on average significantly higher in the case group than benign or healthy control groups. Differences between benign and healthy control groups were not statistically significant (**Table 1**).

Analysis of serum marker levels by pathology, tumor staging, and metastasis of pulmonary carcinomas

Serum levels of CEA, NSE, CYFRA21-1, and HE4 significantly differed with pulmonary carcinoma

Table 3. Comparison of serum marker levels by TNM stage and metastasis of pulmonary carcinomas

Group	n	CEA (ng/mL)	NSE (ng/mL)	CYFRA21-1 (ng/mL)	HE4 (pmol/L)
TNM stage I-II, without metastasis	28	29.78±14.24[c]	21.49±11.22	36.67±43.49	79.65±50.53[c]
TNM stage III-IV, without metastasis	16	34.66±25.93[c]	23.11±16.97	37.54±42.06	111.66±82.89[c]
With metastasis	36	62.22±49.33[a,b]	23.64±16.01	48.13±47.27	227.71±195.57[a,b]
F		6.252	0.193	0.576	17.802
P		0.003	0.825	0.565	< 0.001

[a]$P < 0.05$, vs TNM stage I-II, without metastasis; [b]$P < 0.05$, *vs* TNM stage III-IV, without metastasis; [c]$P < 0.05$, *vs* metastasis.

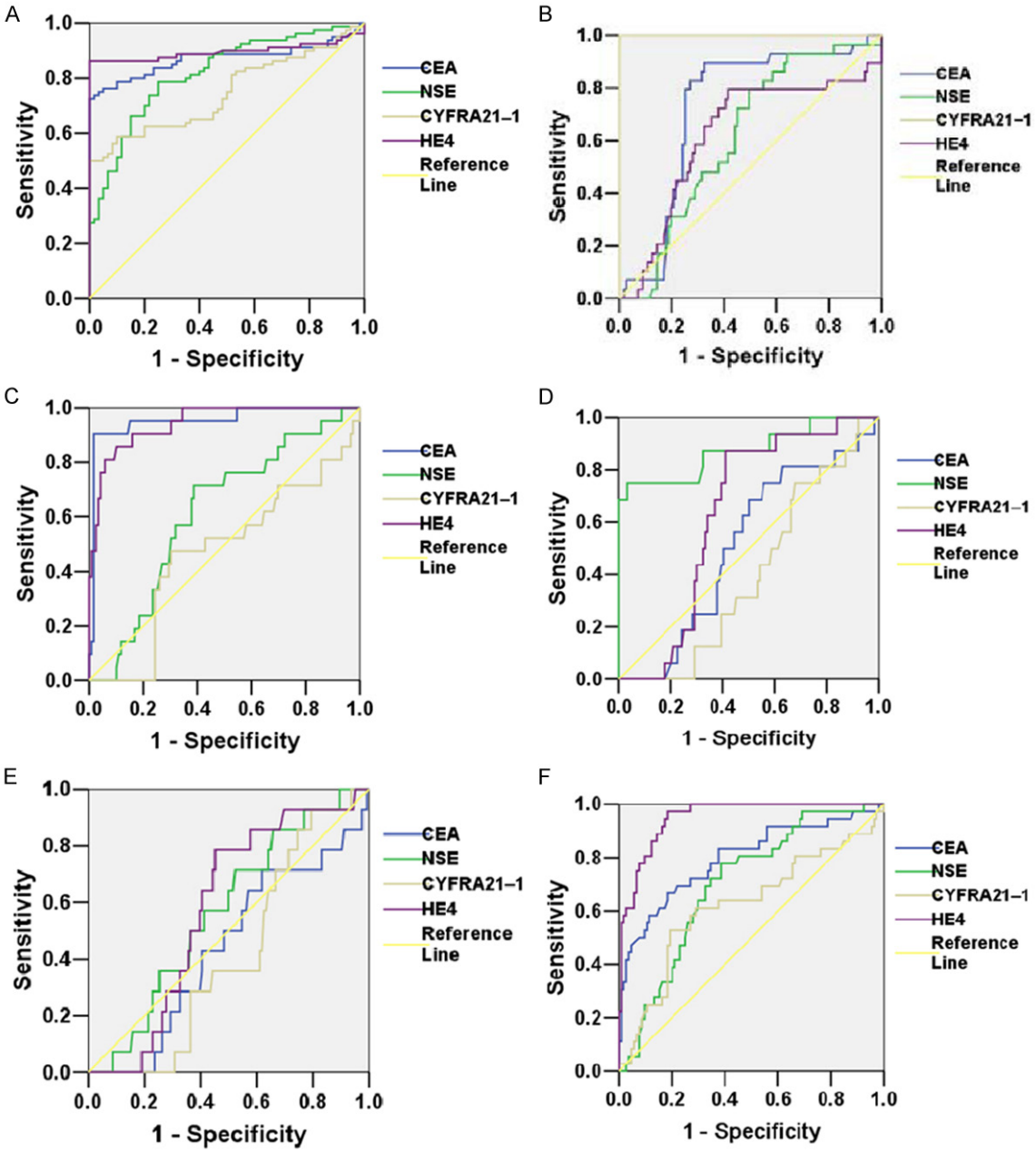

Figure 1. ROC curves of serum markers CEA, NSE, CYFRA21-1, and HE4 for the diagnosis of pulmonary carcinoma. A. Pulmonary carcinoma; B. Squamous carcinoma; C. Adenocarcinoma; D. Small cell lung cancer; E. Undifferentiated carcinoma; F. Carcinoma metastaticum.

Table 4. Comparison of serum marker levels for diagnosing pulmonary carcinoma

Markers	Area	Standard error	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
CEA	0.870	0.033	0.000	0.806	0.934
NSE	0.818	0.035	0.000	0.749	0.888
CYFRA21-1	0.746	0.041	0.000	0.666	0.827
HE4	0.897	0.031	0.000	0.836	0.958

Table 5. Diagnostic value of serum markers for squamous carcinoma

Test result variable	Area	Standard error	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
CEA	0.727	0.049	0.000	0.631	0.823
NSE	0.617	0.052	0.054	0.515	0.718
CYFRA21-1	1.000	0.000	0.000	1.000	1.000
HE4	0.622	0.063	0.043	0.499	0.745

Table 6. Diagnostic value of serum markers for adenocarcinoma

Test result variable	Area	Standard error	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
CEA	0.954	0.027	0.000	0.900	1.007
NSE	0.615	0.061	0.094	0.495	0.734
CYFRA21-1	0.468	0.070	0.636	0.331	0.605
HE4	0.944	0.023	0.000	0.898	0.989

pathologies (F = 31.981, 20.361, 118.612, 19.287, respectively). Specifically, serum CEA and HE4 levels were significantly higher in adenocarcinomas, serum CYFRA21-1 levels were significantly higher in squamous carcinomas, and serum NSE levels were significantly higher in SCLCs than other pathology types (**Table 2**).

CEA and HE4 levels significantly differed among pulmonary carcinoma patients at different TNM stages (F = 6.252) and with metastasis (F = 17.802). Serum CEA and HE4 levels of pulmonary carcinoma patients with metastasis were significantly higher than those of stage TNM I-II patients without metastasis or stage TNM III-IV patients without metastasis. NSE and CYFRA21-1 levels did not significantly differ among pulmonary carcinoma patients at different TNM stages (F = 0.193) and in regards to metastasis (F = 0.576) (**Table 3**).

Diagnostic value of serum markers for pulmonary carcinoma

To assess the diagnostic value of each serum marker for pulmonary carcinoma, we analyzed AUC for ROC curves (**Figure 1A**). Detection of serum marker levels of CEA, NSE, CYFRA21-1, and HE4 was valuable for diagnosing pulmonary carcinoma, as shown in **Table 4**.

Diagnostic value of serum markers for pathology, TNM staging, and metastasis of pulmonary carcinoma

Serum markers CYFRA21-1 (AUC = 1.000), CEA (AUC = 0.727), and HE4 (AUC = 0.622) showed the most promise as diagnostic markers for squamous carcinoma (**Table 5**; **Figure 1B**). CEA (AUC = 0.954) and HE4 (AUC = 0.944) showed the most promise as diagnostic makers for adenocarcinoma (**Table 6**; **Figure 1C**). NSE (AUC = 0.876) showed the most promise as a diagnostic marker for SCLC (**Table 7**; **Figure 1D**). Serum marker levels were not statistically relevant as diagnostic markers for undifferentiated carcinoma (**Table 8**; **Figure 1E**). Serum CEA, NSE, CYFRA21-1, and HE4 levels were also diagnostically valuable for carcinoma metastaticum (**Table 9**; **Figure 1F**).

Discussion

This study indicates that pulmonary carcinoma patients have abnormal expression of serum tumor markers such as HE4, which therefore may be useful for auxiliary diagnosis and evaluation of pulmonary carcinoma. Further, different pulmonary carcinoma pathologies showed stronger correlation with particular serum markers. Adenocarcinoma patients had significantly higher serum levels of CEA and HE4, while squamous carcinoma patients had significantly higher serum levels of CYFRA21-1 and SCLC patients had significantly higher serum

Table 7. Diagnostic value of serum markers for small cell lung cancer

Marker	Area	Standard error	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
CEA	0.511	0.067	0.885	0.380	0.642
NSE	0.876	0.058	0.000	0.762	0.990
CYFRA21-1	0.402	0.059	0.203	0.286	0.518
HE4	0.636	0.053	0.077	0.532	0.740

Table 8. Diagnostic value of serum markers for undifferentiated carcinoma

Marker	Area	Standard error	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
CEA	0.439	0.075	0.455	0.292	0.586
NSE	0.567	0.070	0.410	0.431	0.703
CYFRA21-1	0.414	0.059	0.294	0.298	0.531
HE4	0.577	0.062	0.347	0.455	0.699

Table 9. Diagnostic value of serum markers for metastatic pulmonary carcinoma

Marker	Area	Standard error	Asymptotic significance	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
CEA	0.796	0.048	0.000	0.702	0.889
NSE	0.700	0.047	0.000	0.608	0.793
CYFRA21-1	0.622	0.059	0.030	0.507	0.736
HE4	0.949	0.017	0.000	0.916	0.982

levels of NSE. AUC analysis revealed that serum CYFRA21-1, CEA, and HE4 levels are of potential diagnostic value for squamous carcinoma and that serum CEA and HE4 levels are of potential diagnostic value for adenocarcinoma. AUC analysis also revealed that serum NSE levels are of potential diagnostic value for SCLC.

These results indicate that the examined serum markers are associated with and may be useful in auxiliary diagnosis of pulmonary carcinomas. The diagnostic efficiency of HE4 for adenocarcinoma was relatively high, and the diagnostic efficiency of CYFRA21-1 for squamous carcinoma was highest. Clinically, it is worth mentioning that only NSE was of value for diagnosing SCLC, and malignancy of SCLC was significantly higher than that of NSCLC.

In addition, serum CEA and HE4 levels of pulmonary carcinoma patients with metastasis were significantly higher than those of TNM stage I-II or III-IV patients without metastasis. AUC analysis of serum marker levels revealed that HE4 had the highest diagnostic efficiency for metastatic pulmonary carcinoma, indicating that HE4 may be an auxiliary index for pulmonary carcinoma metastasis and may help evaluate tumor progression.

Among the four serum markers selected for this study, CEA is a polysaccharide protein complex and broad-spectrum tumor marker, as the protein is expressed in multiple tumors and is associated with tumor malignancy. Studies have verified that serum CEA levels of pulmonary carcinoma patients are correlated with *EGFR* mutation rate, so serum CEA levels can guide targeted patient therapies [18]. Serum CEA levels also are closely associated with prognosis of pulmonary carcinoma patients. Increased CEA levels are independently related with decreased postoperative survival rate, and serum CEA levels can predict pulmonary carcinoma patient prognosis after surgical tumor resection. For recurrent advanced pulmonary carcinoma patients whose tissues are inaccessible, serum CEA levels also can predict treatment efficacy of EGFR-TKI drugs [19].

CYFRA21-1 is a new tumor marker associated with malignant epithelial tumorigenesis, such as pulmonary carcinoma, nasopharyngeal carcinoma, esophageal carcinoma, laryngeal carcinoma, urinary bladder carcinoma, mammary adenocarcinoma, oophoroma, and colorectal cancer [20]. Previous studies have verified that serum CYFRA21-1 is related to disease progression and prognosis indicators, such as imageological disease control and progression-free survival of pulmonary carcinoma patients [21]. The marker can provide evidence for early adjustment of therapeutic regimens and can serve as an alternative index to assess chemo-

therapeutic efficacy of patients with progressive pulmonary carcinoma [22].

As a neuron-specific serum marker, NSE can sensitively reflect the degree of neuron impairment among central nervous system injuries, such as acute cerebrovascular disease, epilepsy, acute brain trauma, and neonate hypoxic-ischemic encephalopathy. As a serum tumor marker, NSE is mainly associated with neuroblastoma and SCLC, and it has lower diagnostic efficiency for NSCLC than other tumor markers, such as CEA [23]. Therefore, NSE is often clinically used together with other markers, including CEA, CYFRA21-1, CA199, CA125, and SCC, in joint detection for differential diagnosis, chemotherapy efficacy evaluation, and prognosis evaluation of pulmonary carcinoma patients [24].

HE4 belongs to one of the whey acidic protein four-disulfide core domains and has features of trypsin inhibitors. Multiple studies have verified that HE4 is associated with malignant tumors, such as oophoroma, endometrial carcinoma, pulmonary carcinoma, and urinary bladder carcinoma [25]. Sensitivity and specificity of HE4 are superior to CA125, and the United States Food and Drug Administration has approved use of HE4 as a tumor marker to monitor efficacy and recurrence of ovarian tumors [26]. Relevant studies have verified that, as a pulmonary carcinoma-related serological marker, HE4 can be highly expressed in serum and malignant hydrothorax of pulmonary carcinoma patients and is a useful index for auxiliary diagnosis of early disease. With relatively high sensitivity and specificity, HE4 also might be associated with tumor pathologies and TNM staging. Median total survival time of pulmonary carcinoma patients with low HE4 levels is significantly higher than that of patients with high HE4 levels, so serum HE4 levels may be an independent index to assess prognosis of pulmonary carcinoma patients [27]. In the meantime, joint detection of HE4 and indexes such as CEA, CYFRA21-1, and NSE can significantly improve the efficiency of pulmonary carcinoma diagnosis [28].

This study indicates that serum levels of CEA, NSE, CYFRA21-1, and HE4 are significantly higher in individuals with pulmonary carcinoma than control individuals. AUC of those four serum markers indicate they may be useful for auxiliary diagnosis of pulmonary carcinoma, with HE4 and CEA showing the highest promise as diagnostic markers.

Disclosure of conflict of interest

None.

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