Detection of serum HE4 levels contributes to the diagnosis of lung cancer

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Abstract. Lung cancer (LC) is the most frequently diagnosed cancer and is the leading cause of cancer-associated death. Serum markers that exhibit high sensitivity and specificity for LC may assist in the diagnosis and prognosis of LC. The banked serum samples from 599 individuals, including 201 healthy controls, 124 patients with benign lung diseases, and 274 LC cases, were used. The serum concentrations of biomarkers were determined by electrochemiluminescence immunoassay and chemiluminescence immunoassay. The results showed that the serum human epididymis secretory protein 4 (HE4) levels in the LC group were significantly higher than in the healthy and benign lung disease groups. The serum levels of HE4, NSE, and CYFRA21-1 were significantly higher in patients with LC compared to those in the benign lung disease group. The area under the area under the curve (AUC) of HE4 for discriminating LC from healthy controls was 0.851 (95% CI, 0.818-0.884) and 0.739 (95% CI, 0.695-0.783), 0.747 (95% CI, 0.704-0.790), 0.626 (95% CI, 0.577-0.676), and 0.700 (95% CI, 0.653-0.747) for NSE, CYFRA21-1, SCC, and ProGRP, respectively. The AUC value of the combination of serum HE4 combined with NSE, CYFRA21-1, SCC, and proGRP for cancer diagnosis was 0.896 (95% CI, 0.868-0.923). In early LC, the AUC value of HE4 for discriminating early LC from healthy controls was 0.802 (95% CI, 0.758-0.845), 0.728 (95% CI, 0.679-0.778), 0.699 (95% CI, 0.646-0.752), 0.605 (95% CI, 0.548-0.662), and 0.685 (95% CI, 0.630-0.739) for NSE, CYFRA21-1, SCC, and ProGRP, respectively. The AUC value of the combination of serum HE4 with NSE, CYFRA21-1, SCC, and proGRP for early LC was 0.867 (95% CI, 0.831-0.903). Serum HE4 is a promising LC biomarker, particularly for early-stage LC. Measuring serum HE4 levels may improve the diagnostic efficiency of LC.

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Introduction

Lung cancer (LC), as the most frequently diagnosed cancer, is the leading cause of cancer-associated death, with an estimated 1.8 million deaths (accounting for 18% of all cancer-associated deaths). The 5-year LC survival rate is 10 to 20% in most countries based on patients diagnosed between 2010 and 2014 (1). LC is divided into small cell LC (SCLC) and non-small cell LC (NSCLC). SCLC accounts for 15% of all LC cases, whereas NSCLC accounts for 85% of all cases (2). NSCLC can be further subdivided into adenocarcinoma (AC), squamous cell carcinoma (SC), and large cell carcinoma (3). The major challenge facing the management of LC is that the majority of patients are diagnosed with advanced cancer in the first instance; when diagnosed, >75% of patients are at stage III or IV (4,5). Low-dose CT is a standard method for LC screening, although it has a high false positive rate and carries the risk of potential radiation hazards (6). Serum neuron-specific enolase (NSE), cytokeratin 19 fragment (CYFRA21-1), squamous cell carcinoma antigen (SCC), and progastrin-releasing peptide (proGRP) are widely used biomarkers for LC (7,8). However, the diagnostic efficacy of the aforementioned biomarkers is insufficient for meeting the clinical diagnostic and therapeutic requirements (7). To use biomarkers for clinical conditions, biomarkers that improve the diagnostic efficiency of LC are required.

Human epididymis secretory protein 4 (HE4), glycosylated, acts as an extracellular protease inhibitor (9). While it was discovered in human epididymal tissue cells, HE4 is typically expressed in a variety of normal tissues, including the male reproductive system, respiratory tract, and nasopharynx, amongst others (10). Conversely, HE4 expression is increased in multiple tumor cell lines, such as ovarian, colon, breast,

Table I. Characteristics of the study participants.

Diagnosis	Total, n	Sex, n		Age median (range)
		Male	Female	
Healthy control	201	82	119	52.49 (29-82)
Benign lung diseases	124	66	58	56.47 (29-78)
Lung cancer	274	152	122	59.32 (20-79)
NSCLC	259	141	118	58.64 (20-79)
Stage I	176	76	100	57.30 (20-77)
Stage II	7	7	0	63.28 (56-72)
AC	223	107	116	57.97 (20-79)
SC	36	34	2	62.83 (39-76)
SCLC	15	11	4	63.40 (47-76)

AC, adenocarcinoma; SC, squamous cell carcinoma; SCLC, small cell lung cancer; BLD, benign lung diseases; HC, healthy controls.

lung, and renal cancer (11). As a result, HE4 is frequently studied as a potential biomarker in various tumors.

HE4 was first used in the auxiliary diagnosis of gynecological tumors. For the diagnosis of ovarian epithelial carcinoma, the area under the curve (AUC) of HE4 was 0.92, while the AUC of HE4 combined with CA125 improved to 0.94 (12). Researchers have found that not only can HE4 be used as a screening tool for ovarian cancer, but also as a marker of ovarian cancer recurrence (13,14). As an independent prognostic factor in endometrial cancer, HE4 is positively correlated with advanced lymph node metastasis of endometrial cancer (15). Serum HE4 levels can be used as a marker for the diagnosis of early LC, in which the AUC reached 0.82 (16). The expression of HE4 was notably increased in both advanced LC and node-positive LC groups. The overall survival time with high expression of HE4 was considerably shorter, which indicated that HE4 was an independent prognostic factor of LC (17). Recent research also found that HE4 autoantibodies may be a marker of early LC (18).

The results of the present study confirmed that HE4 had good diagnostic efficiency for LC, particularly for early-stage LC. When HE4 was combined with NSE, CYFRA211, SCC, and ProGRP, the diagnostic efficiency for LC was further improved. These results further add to the body of evidence highlighting the value of HE4 as a marker of LC.

Materials and methods

Patients and healthy controls. The serum samples used in the present study were collected during physical examinations on inpatients at the Department of Thoracic Surgery of Tangshan People's Hospital between January 2020 and May 2022. All volunteers signed an informed consent form. The Ethics Committee at Tangshan People's Hospital approved the collection and use of serum (approval no. RMYY-LLKS-2019-0620-1). Serum was collected from 599 individuals, including 201 healthy controls, 124 patients who were diagnosed with benign lung diseases (BLD), and 274 with LC. The LC samples included 259 NSCLC patients and 15 with SCLC (Table I). The inclusion and exclusion criteria were: The LC patients had pathologically confirmed LC and had no treatment before enrollment; The BLD patients had pathologically confirmed benign diseases; Patients with a history of any type of tumor or multiple tumors were excluded from the study enrollment in the healthy control group, which was defined as individuals who had no evidence of tumors at a recent health checkup and comprehensive health assessment.

Measurements. Approximately 3 ml whole blood was collected and centrifuged for 10 min at 1,500 x g, 20˚C. Serum was collected and stored in a refrigerator at -80˚C for later use. HE4, NSE, CYFRA211, and ProGRP levels were detected using a Roche E601 electrochemiluminescence immunoassay analyzer (Roche Diagnostics), and SCC was detected using an Abbott i2000 chemiluminescence immunoassay analyzer (Abbott Pharmaceutical Co. Ltd.). The HE4, NSE, CYFRA211 and ProGRP detection reagents purchased from Roche Diagnostics, and the SCC detection reagent purchased from Abbott Pharmaceutical Co. Ltd. were calibrated before detection.

Statistical analysis. For data processing, SPSS version 22.0 (IBM Corp.) was used. The measurement data was skewed, thus, a Mann-Whitney U test was used to compare the data between the two groups. A Kruskal-Wallis H test followed by a Dunn's test was used to compare the data between multiple groups. The receiver operating characteristic (ROC) curve was used for the analysis of the diagnostic efficiency. The data are presented as the median and interquartile range (25,75). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Serum HE4 levels are increased in LC patients. The serum HE4 levels in the LC group were significantly higher than that of the healthy and benign lung disease groups (both $P<0.05$). The serum HE4 levels were also significantly higher in the AC, SC, and SCLC subgroups compared with the healthy control group and BLD groups (all $P<0.05$; (Fig. 1A). Furthermore, the association between serum HE4 levels and the clinical

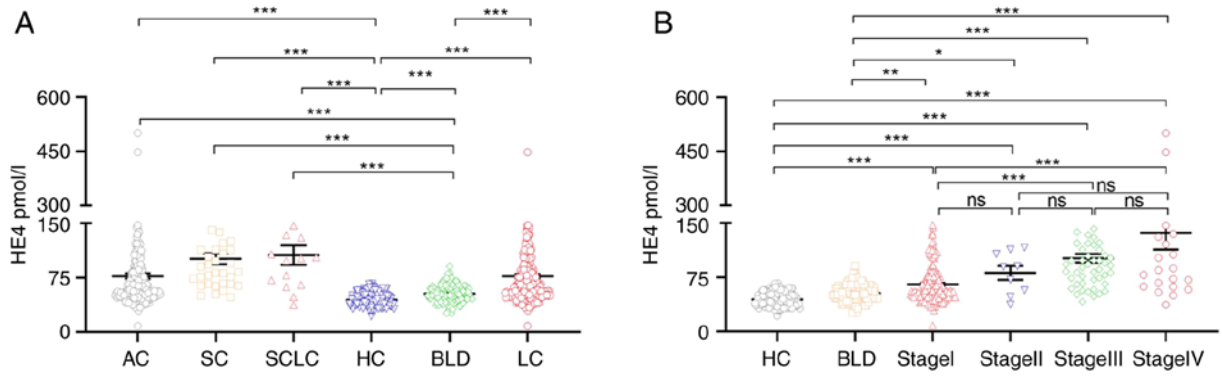

Figure 1. HE4 serum levels in the samples from patients with lung cancer, benign lung disease, and healthy controls. (A) The serum HE4 levels in patients compared by disease status. (B) The serum HE4 levels by disease stage and comparison groups. LC n=274; LC included AC, SC, and SCLC. AC, n=223; SC, n=36; and SCLC, n=15; BLD, n=124. HC, n=201. A Kruskal-Wallis H test followed by a Dunn's test was used to compare the data between HC, BLD, LC, AC, SC, SCLC, stage I, stage II, stage III, and stage IV. *P<0.05, **P<0.01, ***P<0.001. ns, not significant; HE4, human epididymis secretory protein 4; LC, lung cancer; AC, adenocarcinoma; SC, squamous cell carcinoma; SCLC, small cell lung cancer; BLD, benign lung disease.

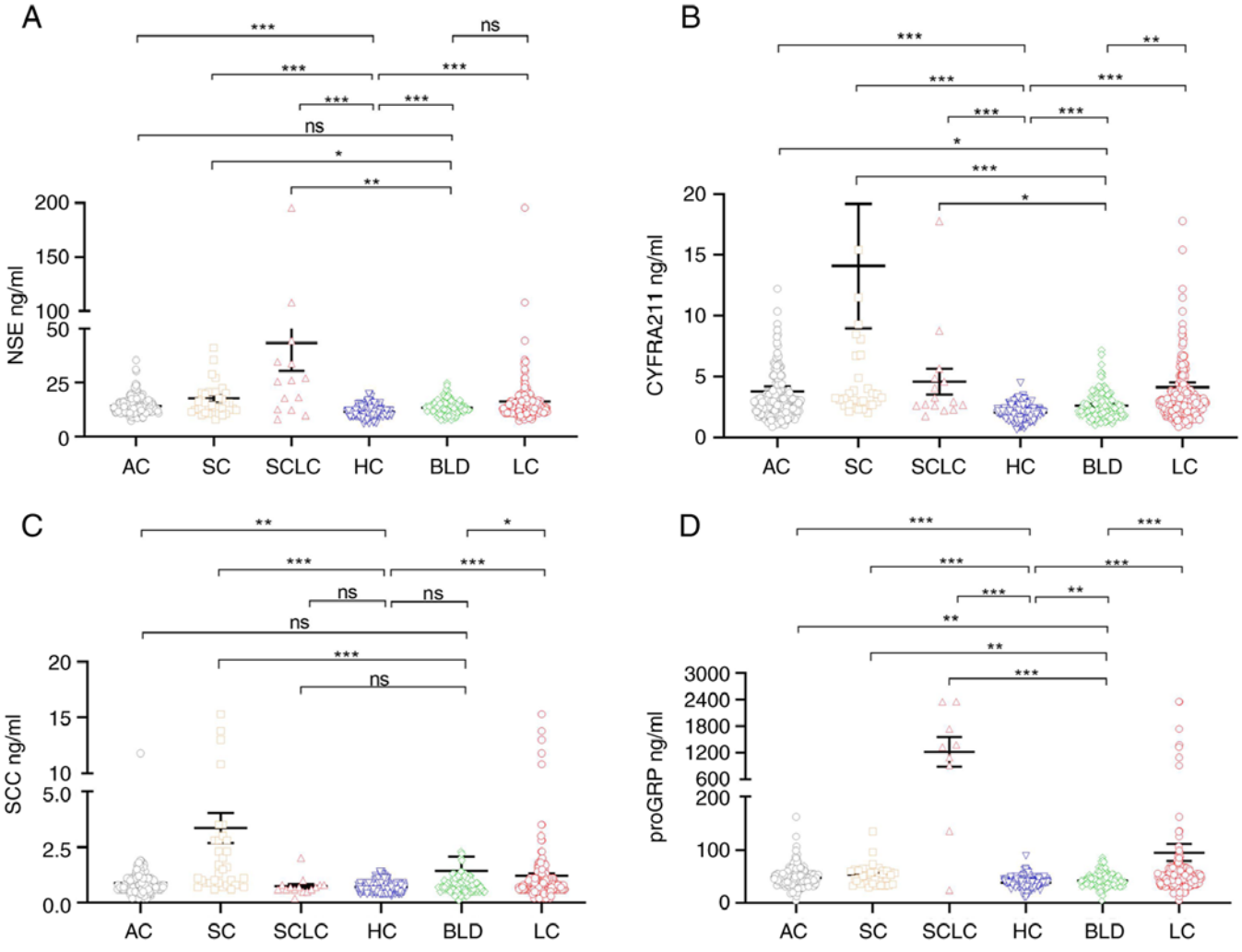

Figure 2. NSE, CYFRA21-1, SCC, and proGRP serum levels in patients with LC and in the HC group. The serum levels of (A) NSE, (B) CYFRA21-1, (C) SCC, and (D) proGRP. A Kruskal-Wallis H test was used to compare the data between HC, BLD, LC, AC, SC, and SCLC. *P<0.05, **P<0.01, ***P<0.001. ns, not significant; AC, adenocarcinoma; SC, squamous cell carcinoma; SCLC, small cell lung cancer; BLD, benign lung diseases; HC, healthy control; NSC, serum neuron-specific enolase; CYFRA21-1, cytokeratin 19 fragments; SCC, squamous cell carcinoma antigen; proGRP, progastrin-releasing peptide.

characteristics of LC cases was assessed. Serum HE4 levels in patients with stage III and IV LC were significantly higher than that in patients with stage I LC (P<0.05; Fig. 1B). Serum HE4 levels were found to be associated with sex (P<0.05), age (P<0.05), tumor size (P<0.05), T stage (P<0.05), N stage (P<0.05), M stage (P<0.05), and AJCC stage III and IV

Table II. Association between serum HE4 levels lung cancer characteristics.

Variable	N	HE4, mol/l[a]	P-value
Sex			0.0001
Male	152	81.33 (63.06-09.90)	
Female	122	54.12 (46.55-65.55)	
Age (years)			0.0001
>60	140	81.53 (59.86-109.90)	
≤60	134	55.59 (46.94-71.73)	
Tumor diameter (cm)			0.0001
≥3	29	90.16 (66.11-113.35)	
<3	175	55.97 (47.45-73.68)	
Unknown	70		
T stage			0.0001
T1	163	56.18 (47.45-75.49)	
T2	69	86.99 (67.95-113.35)	0.0001
T3	16	91.68 (68.35-144.23)	0.0001
T4	11	114.82 (55.42-156.90)	0.0040
Unknown	15		
N stage			0.0001
Positive (N1-3)	78	93.87 (67.01-133.98)	
Negative (N0)	180	56.66 (47.54-76.26)	
Unknown	16		
AJCC stage			0.0001
I	176	56.67 (47.84-76.13)	
II	9	89.07 (52.49-110.25)	0.3200
III	52	93.20 (72.12-121.85)	0.0001
IV	25	101.80 (64.69-160.26)	0.0001
Unknown	12		
Distant metastasis			0.0001
Absent	239	63.20 (50.04-88.57)	
Present	24	104.05 (68.29-168.70)	
Unknown	11		

[a]Median and interquartile range (25,75). A Mann-Whitney U test was used to compare sex, age, tumor size, N stage, and M stage. A Kruskal-Wallis H test followed by a Dunn's test was used to compare T stage and AJCC stage.

(P<0.05) (Table II). Based on these results, serum HE4 may serve as a potential marker of LC.

HE4, NSE, CYFRA21-1, SCC, and proGRP serum concentrations in LC patients and healthy controls. The serum concentrations of HE4, NSE, CYFRA21-1, SCC, and proGRP in the LC, AC, SC, and SCLC patients as well as the healthy controls are shown in Table III. Compared with the healthy control group, the serum HE4, NSE, CYFRA21-1, SCC, and proGRP were significantly increased in the LC, AC, and SC subgroups (P<0.05), but only the serum concentrations of HE4, NSE, CYFRA21-1, and proGRP were markedly higher in SCLC patients (P<0.05), the serum concentrations of SCC were not markedly higher in the SCLC subgroup (P>0.05). Compared with the BLD group, the serum concentrations of HE4, CYFRA21-1, and proGRP were markedly higher than those in the LC, AC, SC, and SCLC groups (P<0.05); the serum concentrations of NSE were markedly higher in the SC and SCLC patients (P<0.05), but was not markedly higher in the LC and AC patients (P>0.05); the serum concentrations of SCC were markedly higher in the LC and SC patients (P<0.05), but was not markedly higher in the AC and SCLC patients (P>0.05). Moreover, HE4 exhibited the most substantial discriminative ability for LC (Figs. 1A and 2A-D).

Value of serum HE4, NSE, CYFRA21-1, SCC, and proGRP for diagnosis of LC. ROC analysis was performed to better understand the diagnostic value of serum HE4, NSE, CYFRA21-1, SCC, and proGRP for LC. The AUC of HE4 for discriminating LC from healthy controls was 0.851 (95% CI, 0.818-0.884) and 0.739 (95% CI, 0.695-0.783), 0.747 (95% CI, 0.704-0.790), 0.626 (95% CI, 0.577-0.676), and 0.700 (95% CI, 0.653-0.747) for NSE, CYFRA21-1, SCC, and proGRP, respectively. (Fig. 3A, Table IV). The cut-off values with a specificity of

Table III. Serum HE4, NSE, CYFRA21-1, SCC, and proGRP levels in the different groups.

		HE4, mol/l		NSE, ng/ml		CYFRA211, ng/ml		SCC, ng/ml		proGRP, pg/ml	
Diagnosis	n	Median	P25, P75	Median	P25, P75	Median	P25, P75	Median	P25, P75	Median	P25, P75
Healthy control	201	41.73	36.99, 51.23	11.18	9.54, 13.12	2.01	1.55, 2.49	0.7	0.50, 0.90	38.65	33.87, 43.02
Benign lung diseases[a]	124	52.56	45.39, 61.19	13.17	11.54, 15.11	2.32	1.79, 3.23	0.8	0.60, 0.90	42.09	33.97, 48.72
Lung cancer[b]	274	65.71	51.49, 97.94	13.71	11.51, 16.43	2.91	2.11, 3.85	0.8	0.60, 1.10	47.23	36.75, 58.31
NSCLC[c]	259	65.23	51.22, 91.94	13.62	11.47, 16.24	2.91	2.09, 3.83	0.8	0.60, 1.10	46.59	36.08, 56.37
Stage I[d]	176	56.6	47.80, 76.17	13.35	11.57, 15.54	2.62	1.89, 3.36	0.8	0.60, 1.00	45.96	36.86, 54.40
Stage II[e]	7	89.07	56.89, 113.30	16.24	13.37, 19.85	3.64	2.95, 11.49	0.8	0.60, 1.30	49.38	33.00, 54.29
AC[f]	223	60.54	49.23, 86.70	13.58	11.45, 15.87	2.81	1.98, 3.58	0.8	0.60, 1.00	45.97	36.12, 55.04
SC[g]	36	89.31	66.31, 120.25	14.78	11.62, 20.39	3.59	2.77, 9.09	1.55	0.90, 3.38	52.7	36.83, 61.09
SCLC[h]	15	101.8	64.95, 133.70	25.98	12.54, 44.39	2.93	2.61, 4.88	0.7	0.60, 0.80	911.2	372.70, 1,738.00

[a]Comparison between benign lung diseases and controls: HE4, P<0.0001; NSE, P<0.0001; CYFRA21-1, P<0.0001; SCC, P=0.058; proGRP, P=0.006. [b]Comparison between LC and controls: HE4, P<0.0001; NSE, P<0.0001; CYFRA21-1, P<0.0001; SCC, P<0.0001; ProGRP, P<0.0001. [c]Comparison between NSCLC and controls: HE4, P<0.0001; NSE, P<0.0001; CYFRA21-1, P<0.0001; SCC, P<0.0001; proGRP, P<0.0001. [d]Comparison between Stage I LC and controls: HE4, P<0.0001; NSE, P<0.0001; CYFRA21-1, P<0.0001; SCC, P<0.0001; proGRP, P<0.0001. [e]Comparison between Stage II LC and controls: HE4, P<0.0001; NSE, P<0.0001; CYFRA21-1, P<0.0001; SCC, P=0.169; proGRP, P=0.043. [f]Comparison between AC and controls: HE4, P<0.0001; NSE, P<0.0001; CYFRA21-1, P<0.0001; SCC, P=0.001; proGRP, P<0.0001. [g]Comparison between SC and controls: HE4, P<0.0001; NSE, P<0.0001; CYFRA21-1, P<0.0001; SCC, P<0.0001; proGRP, P<0.0001. [h]Comparison between SCLC and controls: HE4, P<0.0001; NSE, P<0.0001; CYFRA211, P<0.0001; SCC, P=0.866; proGRP, P<0.0001. A Kruskal-Wallis H test was used to compare the data between the controls and Benign lung diseases, LC, NSCLC, Stage I LC, Stage II LC, AC, SC, and SCLC groups. AC, adenocarcinoma; SC squamous cell carcinoma; SCLC, small cell lung cancer; NSCLC, non SCLC; BLD, benign lung diseases; HC, healthy control; NSC, serum neuron-specific enolase; CYFRA21-1, cytokeratin 19 fragments; SCC, squamous cell carcinoma antigen; proGRP, progastrin-releasing peptide.

Table IV. Sensitivity and specificity of biomarkers alone or combined for the diagnosis of LC.

Type	AUC	95% CI	Cut-off	Sensitivity (%)	Specificity (%)	Youden index
Lung cancer						
HE4	0.851	0.818-0.884	60.14	57.4	95.0	0.524
NSE	0.739	0.695-0.783	16.01	28.3	95.0	0.233
CYFRA211	0.747	0.704-0.790	3.14	40.1	95.0	0.351
SCC	0.626	0.577-0.676	1.11	21.7	95.0	0.167
proGRP	0.700	0.653-0.747	54.20	32.7	95.0	0.277
Combined	0.896	0.868-0.923	0.74	64.0	95.0	0.590
Adenocarcinoma						
HE4	0.826	0.787-0.864	60.15	50.5	95.0	0.455
NSE	0.727	0.680-0.775	16.01	23.6	95.0	0.187
CYFRA211	0.717	0.668-0.765	3.14	36.4	95.0	0.314
SCC	0.597	0.543-0.651	1.11	16.8	95.0	0.118
proGRP	0.682	0.631-0.733	54.21	27.3	95.0	0.223
Combined	0.878	0.846-0.910	0.73	58.2	95.0	0.532
Squamous carcinoma						
HE4	0.972	0.944-0.999	60.15	88.9	95.0	0.839
NSE	0.772	0.683-0.860	16.04	41.7	95.0	0.367
CYFRA211	0.914	0.868-0.961	3.15	61.1	95.0	0.561
SCC	0.866	0.798-0.934	1.11	55.6	95.0	0.506
proGRP	0.748	0.645-0.852	54.20	44.4	95.0	0.395
Combined	0.996	0.991-1.000	0.14	97.2	95.0	0.922
Small-cell lung carcinoma						
HE4	0.926	0.826-1.000	60.15	86.7	95.0	0.817
NSE	0.842	0.695-0.990	16.04	73.3	95.0	0.684
CYFRA211	0.852	0.755-0.950	3.15	46.7	95.0	0.417
SCC	0.482	0.342-0.623	1.11	6.7	95.0	0.017
proGRP	0.939	0.824-1.000	54.22	93.3	95.0	0.884
Combined	1.000	1.000-1.000	0.00	100.0	95.0	0.950

AC, adenocarcinoma; SC squamous cell carcinoma; SCLC, small cell lung cancer; BLD, benign lung diseases; HC, healthy control; NSC, serum neuron-specific enolase; CYFRA21-1, cytokeratin 19 fragments; SCC, squamous cell carcinoma antigen; proGRP, progastrin-releasing peptide; CI, confidence interval; AUC, area under the curve.

95% were 60.14 pmol/l for HE4, 16.01 μg/l for NSE, 3.14 μg/l for CYFRA21-1, 1.11 μg/l for SCC, and 54.20 ng/l for proGRP. Furthermore, the AUC for serum HE4 combined with NSE, CYFRA21-1, SCC, and proGRP for cancer diagnosis was 0.896 (95% CI, 0.868-0.923).

Next, the diagnostic efficacy of serum HE4, NSE, CYFRA21-1, SCC, and proGRP for AC, SC, and SCLC were analyzed. For AC, the AUC of HE4 for discriminating AC from healthy controls was 0.826 (95% CI, 0.787-0.864). The cut-off values with a specificity of 95% were 60.15 pmol/l for HE4. The AUC value of the combination of serum HE4 with NSE, CYFRA21-1, SCC, and proGRP for cancer diagnosis was 0.878 (Fig. 3B, Table IV).

HE4 had the best diagnostic efficacy for SC. The AUC of HE4 for discriminating SC from healthy controls was AUC 0.972 (95% CI, 0.944-0.999). The cut-off values with a specificity of 95% were 60.15 pmol/l for HE4. The AUC value of the combination of serum HE4 with NSE, CYFRA21-1, SCC, and proGRP for cancer diagnosis was 0.996 (Fig. 3C, Table IV).

proGRP had the best diagnostic efficacy for SCLC. The AUC of proGRP for discriminating SCLC from healthy controls was 0.939 (95% CI, 0.824-1.000). The cut-off values with a specificity of 95% were 54.22 pg/ml for proGRP. The AUC value of the combination of serum HE4 with NSE, CYFRA21-1, SCC, and proGRP for cancer diagnosis was 1.000 (Fig. 3D, Table IV).

Diagnostic value of serum HE4, NSE, CYFRA21-1, SCC, and proGRP for early LC. ROC analysis was performed to assess the diagnostic value of serum HE4, NSE, CYFRA21-1, SCC, and proGRP for early LC. A total of 183 early LC specimens were included (Table III), including 176 patients with stage I LC and 7 patients with stage II LC. No limited-stage SCLCs were collected. The results showed that the AUC values for discriminating early LC from healthy controls were 0.802 (95% CI, 0.758-0.845) for HE4, 0.728 (95% CI, 0.679-0.778) for NSE, 0.699 (95% CI, 0.646-0.752) CYFRA21-1, 0.605 (95% CI, 0.548-0.662) for SCC, and 0.685 (95% CI, 0.630-0.739) for

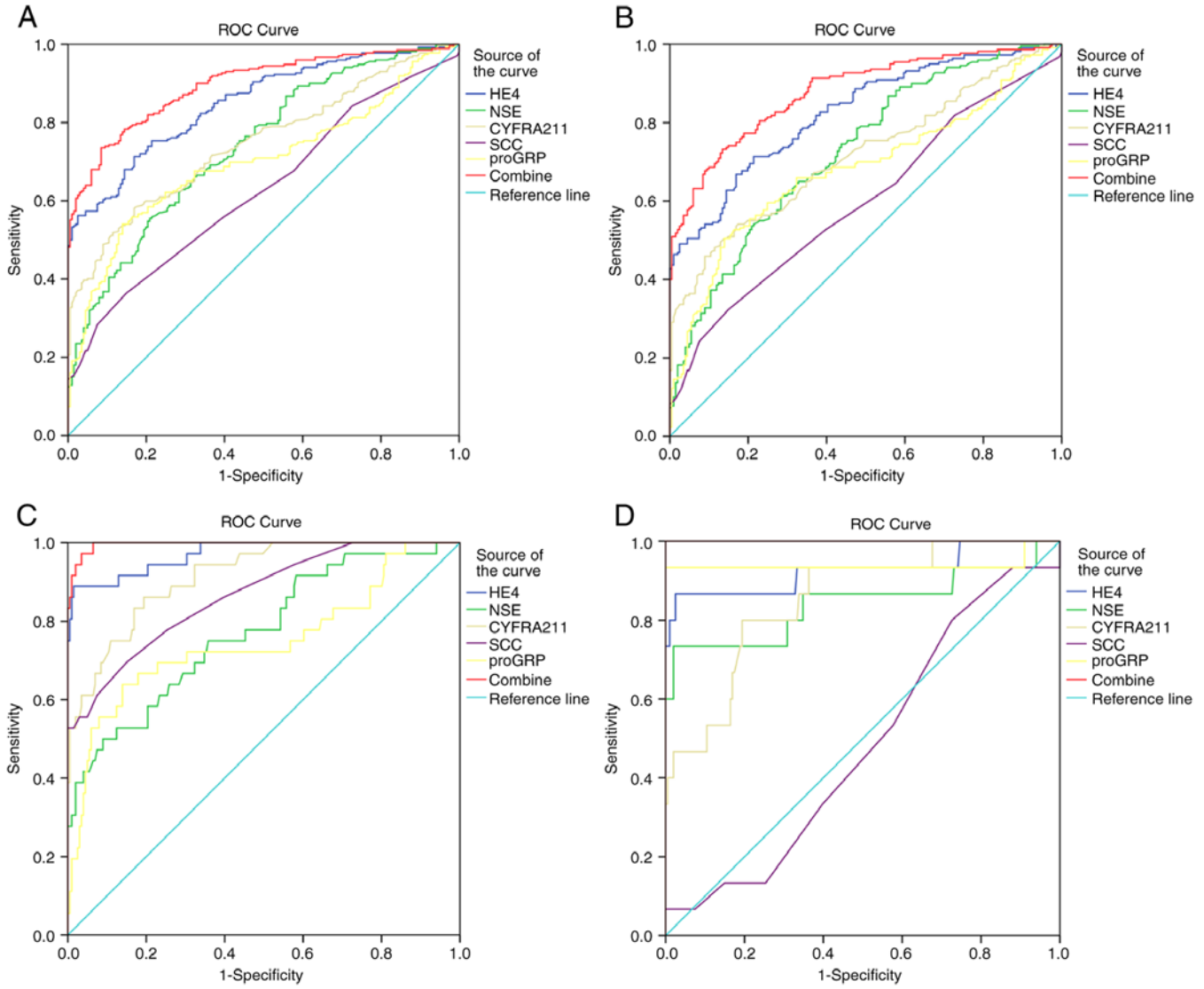

Figure 3. Sensitivity and specificity of biomarkers alone or combined in the diagnosis of lung cancer. The sensitivity and specificity of biomarkers (HE4, NSE, CYFRA21-1, SCC, proGRP) alone or combined for the diagnosis of (A) all lung cancer patients, (B) AC, (C) SC, and (D) SCLC was analyzed by determining the area under the ROC curve. AC, adenocarcinoma; SC, squamous cell carcinoma; SCLC, small cell lung cancer; BLD, benign lung diseases; HC, healthy control; NSC, serum neuron-specific enolase; CYFRA21-1, cytokeratin 19 fragments; SCC, squamous cell carcinoma antigen; proGRP, progastrin-releasing peptide.

proGRP (Fig. 4, Table V). The cut-off value with a specificity of 95% was 60.15 pmol/l for HE4, 16.01 μg/l for NSE, 3.14 μg/l for CYFRA21-1, 1.11 μg/l for SCC, and 54.20 ng/l for proGRP. Furthermore, the AUC value of combined serum HE4 with NSE, CYFRA21-1, SCC, and proGRP for cancer diagnosis was 0.867. The above results indicated that serum HE4 may serve as a biomarker of early LC and significantly improve diagnostic efficiency of early LC.

Discussion

The worldwide incidence and mortality of LC have been reported to be 56.3 per 100,000 population and 35.0 per 100,000 population, respectively (19), and identifying biomarkers to improve the diagnostic efficiency is an important research question. The results of the present study indicated that serum HE4 effectively improved the diagnostic efficiency of LC, and serum HE4 had good diagnostic efficiency for early LC. These findings are consistent with previous research. Iwahori *et al* (20) reported that the AUC of serum HE4 in the diagnosis of LC was 0.988 with a cut-off value of 6.56 ng/ml (sensitivity, 89.8%; specificity, 100%). Liu *et al* (21) found that the AUC of serum HE4 for LC diagnosis was 0.85 with a cut-off value of 77.48 pmol/ml (sensitivity, 67.9%; specificity 93.4). Nagy *et al* (22) found that the AUC of serum HE4 for LC diagnosis was 0.848 with a cut-off value of 97.6 pmol/l (specificity, 64.3%; sensitivity, 95.9%). In the present study, the AUC of serum HE4 for LC was 0.851 with a cut-off value of 60.14 pmol/ml (sensitivity, 57.4%; specificity, 95%).

SCC, CYFRA211, and NSE, proGRP are commonly used to diagnose SC and SCLC. Liu *et al* (23) reported that theAUC of serum SCC and CYFRA211 for diagnosing lung squamous cell carcinoma were 0.691 and 0.788. Du *et al* (24) reported that the AUC of proGRP for the diagnosis of SCLC were 0.855 and 0.905. In the present study, the diagnostic efficiency of serum HE4 (0.972) was better than that of serum SCC (0.866)

Table V. Sensitivity and specificity of biomarkers alone or combined for the diagnosis of TNM stage I and II LC patients.

Marker	AUC	95% CI	Cut-off	Sensitivity (%)	Specificity (%)	Youden's index
HE4	0.802	0.758-0.845	60.15	45.1	95.0	0.401
NSE	0.728	0.679-0.778	16.01	22.0	95.0	0.170
CYFRA211	0.699	0.646-0.752	3.14	31.3	95.0	0.263
SCC	0.605	0.548-0.662	1.11	14.8	95.0	0.099
proGRP	0.685	0.630-0.739	54.20	25.8	95.0	0.208
Combined	0.867	0.831-0.903	0.73	52.7	95.0	0.478

LC, lung cancer; HE4, human epididymis secretory protein 4; NSC, serum neuron-specific enolase; CYFRA21-1, cytokeratin 19 fragments; SCC, squamous cell carcinoma antigen; proGRP, progastrin-releasing peptide; CI, confidence interval.

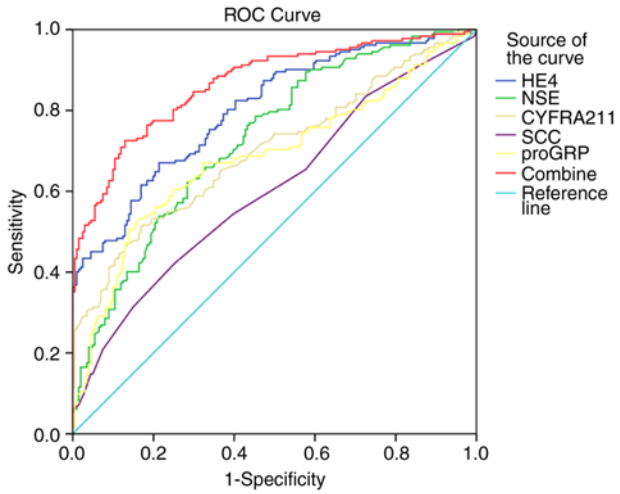

Figure 4. Sensitivity and specificity of biomarkers alone and combined for the diagnosis of TNM stage I and II LC. LC, lung cancer.

and CYFRA211 (0.914) for SC. The diagnostic efficiency of serum proGRP (0.939) was better than that of NSE (0.842) and serum HE4 (0.926) for SCLC. In the present study, the high diagnostic efficiency of SCC, CYFRA211, NSE, and proGRP for SC and SCLC may have been due to the small number of patients and the prevalence of advanced-stage disease. The high diagnostic efficacy of serum HE4 for AC may be related to the high proportion of cases in LC.

Next, the diagnostic efficacy of serum HE4 for early LC was investigated. The results showed that serum HE4 was the best specificity marker for early LC, with a cut-off value of 60.15 pmol/l (sensitivity: 45.1%, specificity: 95.0%), similar to the results reported by Zeng *et al* (16). HE4, combined with serum SCC, CYFRA211, NSE, and proGRP, may further improve the diagnostic efficiency of early LC.

In the present study, the association between serum HE4 and clinicopathological features of LC was analyzed, and it was found that serum HE4 was associated with sex, age, tumor size, T stage, M stage, and AJCC stage. It has been reported that serum HE4 in LC patients is associated with age and sex. Serum HE4 gradually increased with age, and there was a significant difference in levels between males and females (25,26). Previously, it was shown that serum HE4 was positively correlated with age in patients with endometrial cancer (27). This characteristic of HE4 expression requires us to be more careful when interpreting these results, the differences between sexes regarding HE4 levels may be related to its tissue source; specifically high HE4 expression in endometrial tissue may lead to the differences between sexes observed here (28). Whilst the correlation between HE4 and age may be related to its own function, it has been reported that HE4 can promote the proliferation, invasion, and metastasis of endometrial cancer cell lines (29), and HE4 may have a similar effect in LC. The incidence of LC has increased in recent years, and with an increase in age, the risk of developing lung cancer increases, which may lead to the association with the observed regarding HE4 levels. However, the specific mechanistic differences caused by the correlation between HE4, sex, and age remain to be further studied. This suggests that different reference intervals should be formulated according to age and sex when applying serum HE4 as a marker for the diagnosis of LC. The present study found that the larger the tumor diameter, the higher the T and M stage were and that the higher the AJCC grade was, the higher the serum HE4 levels were, which indicates that serum H4 may be associated with a poor prognosis in patients with LC (17).

In conclusion, serum HE4 is a promising biomarker for LC. Several studies have reported that serum HE4 can be used as a marker for the diagnosis and prognosis of LC. The present study further confirmed that serum HE4 could effectively improve the diagnostic efficiency of LC.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JL, YZL and LH conceived and designed the present study, and acquired, analyzed, and interpreted the data. JL and YFL drafted the manuscript. QG performed the experiments. AL, SH, HL, RS, YZ, YFL and XL analyzed the data. JL and LH confirms the authenticity of all raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All volunteers signed an informed consent form. The Ethics Committee at Tangshan People's Hospital approved the collection and use of serum (approval no. RMYY-LLKS-2019-0620-1; Tangshan, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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