doi:10.4149/neo_2022_220207N144

New insights into the diagnostic characteristics and clinical application of serum biomarkers for lung cancer, and human epididymis protein 4 as a new biomarker?

Ming LI[1,#], Yi ZHANG[2,#], Li JIANG[3], Yan LI[4], Gang LI[5], Jianping ZHOU[6], Chen YANG[7], Xinhui LI[8], Wei QU[9], Yong CHEN[10], Qing CHEN[10], Shukui WANG[11,*], Jinliang XING[12,*], Huayi HUANG[10,13,*]

[1]Department of Laboratory Diagnostics, The First Affiliated Hospital of University of Science and Technology of China, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, China; [2]Department of Laboratory Medicine, Qilu Hospital of Shandong University, Jinan, Shandong, China; [3]Department of Laboratory Medicine, Sichuan Provincial Key Laboratory for Human Disease Gene Study, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, Sichuan, China; [4]Department of Laboratory Medicine, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [5]Department of Laboratory Medicine, Henan Provincial People's Hospital, Zhengzhou, Henan, China; [6]Radioimmunoassay Center, Shaanxi Provincial People's Hospital, Xi'an, Shaanxi, China; [7]Department of Laboratory Medicine, Suzhou Hospital Affiliated to Nanjing Medical University, Suzhou, Jiangsu, China; [8]Department of Nuclear Medicine, Xiangya Hospital, Central South University, Changsha, Hunan, China; [9]Department of Nuclear Medicine, Nanjing First Hospital, Nanjing Medical University, Nanjing, Jiangsu, China; [10]Division of In Vitro Diagnostics, Shenzhen Mindray Bio-Medical Electronics Corporation, Shenzhen, Guangdong, China; [11]Department of Laboratory Medicine, Nanjing First Hospital, Nanjing Medical University, Nanjing, Jiangsu, China; [12]State Key Laboratory of Cancer Biology, Department of Physiology and Pathophysiology, Fourth Military Medical University, Xi'an, Shaanxi, China; [13]Department of Surgical Oncology, Roswell Park Comprehensive Cancer Center, Buffalo, New York, United States

**Correspondence: SK_Wang@njmu.edu.cn; Xingjl@fmmu.edu.cn; Huayi.Huang@Roswellpark.org*
[#]Contributed equally to this work.

Received February 7, 2022 / Accepted April 4, 2022

The value of serum tumor biomarkers used for lung cancer diagnosis is still controversial in clinical practice. This study aimed to further dissect and evaluate the clinical value of serum progastrin-releasing peptide (ProGRP), neuron-specific enolase (NSE), squamous cell carcinoma antigen (SCC-Ag), carcinoembryonic antigen (CEA), cytokeratin-19 fragment (CYFRA21-1) together with a potential new biomarker, the human epididymis protein 4 (HE4) for lung cancer diagnosis, in a large cohort of a Chinese population. Ostensibly healthy individuals, as well as those with benign non-cancerous diseases, benign tumors, lung cancers, and other types of malignancies, were enrolled in the study. Serum ProGRP, NSE, SCC-Ag, CEA, CYFRA21-1, and HE4 were analyzed using the chemiluminescence immunoassay. Data were analyzed utilizing the SPSS and GraphPad Prism software. Detailed dissection of the diagnostic characteristics of serum 6 biomarkers on lung cancer was performed. All 6 biomarkers showed capabilities in characterizing lung cancer from other diseases. ProGRP and NSE were highly specific to small cell lung cancer (SCLC); SCC-Ag was a fair biomarker for NSCLC, specifically SCC histotype; CEA showed specificity to SCLC, followed by NSCLC; CYFRA21-1 was a good biomarker for both SCLC and NSCLC; HE4 showed high specificity to SCLC. For NSCLC characterization, CYFRA21-1+HE4+CEA was the best combinatory pattern in the terms of diagnostic performance (AUC=0.8110). The best combinatory analysis for SCLC was ProGRP+NSE+HE4 (AUC=0.9282). Patients with advanced stage, larger tumor, males, and age 50 or older had higher serum biomarkers levels than those with early stage, smaller tumor, females, and age under 50. Six biomarkers had capabilities in characterizing lung cancer with high or fair diagnostic performance. HE4 is a potential biomarker for both SCLC and NSCLC diagnosis, which merits further investigation.

Key words: lung cancer; serum biomarkers; tumor histological type; human epididymis protein 4 (HE4); diagnostic performance

Lung cancer is the most common malignancy worldwide [1]. Despite continuous study and progress made in both diagnoses and treatments, the 5-year survival rates remain low. Currently, low-dose computed tomography (LDCT) is a common and accessible tool for lung cancer diagnosis in clinics. However, the shortness of LDCT is that it has a high false-positive rate, limiting its efficacy in helping to characterize cancer from benign nodules [2]. There is therefore a need to pursue more efforts on a minimally invasive, convenient, and easy to access method – the serum biomarker to

help the screening, diagnoses, therapeutic monitoring, and prognostication of lung cancer [3–5]. Numerous reports have indicated that some serum biomarkers had a fair diagnostic or prognostic performance for lung cancer. In addition, the elevation of these biomarkers could serve as a sign of distant metastasis [6,7]. Nevertheless, reports concerning the diagnostic performance of serum biomarkers on lung cancer were still inconsistent and debatable, thus the biomarkers are underutilized in clinical practice in general. Furthermore, the elevation of some of the biomarkers was found not only in lung cancer but also, they were detected abnormally in other diseases or conditions, making the specificity of the proposed biomarkers more concerned [8–12]. It is unclear whether gender and age have an impact on serum levels of lung cancer biomarkers, thereby interfering with the judgment in clinics. There is still a debate on their ability to characterize histological types of the tumor, therefore, it is still unclear what biomarkers are specific to what histological type of lung cancer. The combinatory detection of biomarkers and what represents the best combinatory pattern to be used in clinical practice, balancing diagnostic power and cost, is also an issue [13]. Furthermore, the sensitivity of biomarkers used in lung cancer screening is generally low. In one study, only 8 out of 47 patients with malignant solitary pulmonary nodules had elevated CEA levels that belonged to lung adenocarcinoma [13]. Thus, evaluations of new potential biomarkers for lung cancer are needed for a better diagnosis, therapeutic monitoring, and prognostication of lung cancer. Human epididymis protein 4 (HE4) is considered an ovarian cancer biomarker used in clinics [14]. However, there are some hints indicating an elevation of HE4 in the serum of lung cancer patients. Therefore, it might be a new biomarker for this malignancy and requires further investigation [15–18]. Here, we further dissect and evaluate the diagnostic performance of a 6-biomarkers panel of lung cancer, specifically, progastrin-releasing peptide (ProGRP), neuron-specific enolase (NSE), squamous cell carcinoma antigen (SCC-Ag), carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA21-1), and human epididymis protein 4 (HE4) from a geographically-based multi-center study in China.

Patients and methods

Study design and ethical approval. This study enrolled lung cancer patients, apparently healthy subjects, patients with pulmonary infections, benign tumors, other benign non-malignant diseases, and other malignancies. All patients enrolled in this study were in pre-treatment. The study was carried out under the approval of the Institutional Review Board (IRB)/Ethics Committee of all participating hospitals. Patients were provided written informed consent on the purpose of the study.

Study site selection. Nine large Tier-3 Class A hospitals in China were selected, representing North (Zhengzhou), Northwest (Xi'an), Southwest (Chengdu), Central (Wuhan), Central South (Changsha), and East China (Jinan, Hefei, Nanjing, and Suzhou) for geographical representation and patient distribution.

Diagnosis of diseases and pathology diagnosis of tumors. The guidelines for clinical diagnosis and treatment of lung cancer (Chinese Medical Association, 2019 edition) were followed for lung cancer diagnosis. For the healthy subject enrollment, the criteria from CLSI EP28-A3C were followed which was also applied for the establishment of a reference interval [19]. For pathology histological analysis, lung tissue specimens were resected surgically, by fiber-optic bronchoscopy or CT-guided puncture. Tissues were fixed with 10% formalin buffer and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E). The diagnosis was made by a pathologist following routine pathological procedures.

The associated diagnostic guidelines were followed for the diagnosis of other non-lung cancer patients including pulmonary infections, benign tumors, other benign non-tumor diseases, and other malignancies in the routine clinical practice. Detailed patients' demographics are listed in Table 1 and Suppl. Table S1.

Sample collection and storage. For ostensibly healthy subjects, fasting blood was collected from all individuals visiting the health examination center of a participating hospital who met the requirements of the study questionnaire following CLSI guidelines [19]. For lung cancer patients, fasting blood was collected the following day of admission as baseline level testing. A serum collecting tube, routinely used in each participating hospital, was used to collect the blood, and the samples were transported to the clinical laboratory for processing by a qualified technician to isolate the serum. The collected serum was then stored at –80 °C for a period of 1–3 months until required.

Chemiluminescent immunoassay of tumor biomarkers. ProGRP (Lot No. 20180101), NSE (Lot No. 20190208), SCC-Ag (Lot No. 20180801), CEA (Lot No. 20190208), CYFRA21-1 (Lot No. 20190209), and HE4 (Lot No. 20190801) were analyzed on a Mindray CL-2000i or CL-6000i Chemiluminescent immunoassay platform (Mindray Bio-Medical Electronics Corporation, Shenzhen, Guangdong, China) following the manufacturer's instructions. Results were deposited in the Laboratory Information System to be further analyzed.

Statistical analyses. According to the CLSI C28-A3 guidelines and the principle of statistics [19], the distribution of the serum levels of 6 individual lung cancer biomarkers from 9 participating hospitals was analyzed by the normality test prior to further analysis. Specifically, statistical analysis of data was performed using the SPSS version 26.0 and GraphPad Prism version 9.0 software. The One-sample Kolmogorov-Smirnov test was used for the data normality test. The Mann-Whitney U-test and the Kruskal-Wallis test were used for data comparison. Graphic production was performed by utilizing GraphPad Prism. The Receiver Operator Charac-

teristic (ROC) curves and associated parameters were calculated by GraphPad Prism. A p-value <0.05 was considered significant difference. Statistical analysis was performed and verified by two statisticians and agreed on the final results.

Results

Demographics and diagnoses of patients. In this study, 1,571 lung cancer patients (1,056 males and 515 females, aged 21–90), 2,259 ostensibly healthy individuals (990 males and 1,269 females, aged 13–87), 388 pulmonary infections (228 males and 160 females, aged 15–92), 84 benign tumors (19 males and 65 females, aged 22–79), 128 other benign non-tumors diseases (75 males and 53 females, aged 14–92), and 83 other malignancies (45 males and 38 females, aged from 22–90) were enrolled, as shown in Table 1 and Suppl. Table S1.

Normality test results of serum levels of 6 lung cancer biomarkers (One-sample Kolmogorov-Smirnov test). Normality test results revealed that all data of serum 6 biomarkers were skewed as shown in Suppl. Figure S1, thus, non-parametric statistical methods were used for all data analysis.

Serum ProGRP, NSE, SCC-Ag, CEA, CYFRA21-1, and HE4 levels in different patient groups and healthy controls (Kruskal-Wallis test). Serum ProGRP, NSE, SCC-Ag, CEA, CYFRA21-1, and HE4 levels were compared among different patient groups (SCLC, NSCLC, pulmonary infections, benign tumors, other benign non-tumor diseases (OBNTD), other malignancies), and healthy controls. Serum levels of biomarkers from all groups were sub-classified into 1-4 subsets based on their concentrations from low to high. The difference among subsets (columns) was significant ($p<0.05$). Serum ProGRP and NSE levels in SCLC were significantly higher than in other patient groups and healthy controls ($p<0.05$, for all). SCC-Ag levels in NSCLC were significantly higher than in other patient groups and in healthy controls ($p<0.05$, for all). The highest CEA levels were seen in SCLC, followed by NSCLC patients ($p<0.05$, compared with other groups). CYFRA21-1 in SCLC and NSCLC were significantly higher than in other patient groups and in healthy controls ($p<0.05$, for all). Significantly increased HE4 was observed in SCLC, followed by NSCLC and pulmonary infections ($p<0.05$). Figure 1 and Suppl. Table S2 display the results described above. For a more detailed comparison among subsets of each biomarker, refer to Suppl. Table S2.

Serum ProGRP, NSE, SCC-Ag, CEA, CYFRA21-1, and HE4 levels in different histological types of lung cancer (Kruskal-Wallis test). As seen in Figure 2A, serum ProGRP and NSE concentrations in SCLC patients are significantly higher than in NSCLC patients ($p<0.0001$ for both); SCC-Ag levels in NSCLC are significantly higher than in SCLC patients ($p=0.002$); further stratified analysis revealed that SCC-Ag was specific to SCC histologic type in NSCLC (Figure 2B). There are no significant differences in CEA

Table 1. Patient demographics.

Groups	n	Age Median (range)
Lung cancers	1571	63 (21–90)
Gender		
M	1056	
F	515	
Age		
<50	155	
≥50	1416	
Histology		
Adenocarcinoma	874	
Squamous cell carcinoma	411	
Small cell lung cancer	197	
Other large cell lung cancer	89	
Clinical stage		
I+II	428	
III+IV	1004	
Healthy controls	2259	52 (13–87)
Gender		
M	990	
F	1269	
Age		
<50	932	
≥50	1327	
Pulmonary infections	388	61 (15–92)
Gender		
M	228	
F	160	
Age		
<50	91	
≥50	297	
Benign tumors	84	52 (22–79)
Gender		
M	19	
F	65	
Age		
<50	33	
≥50	51	
OBNTD	128	63 (14–92)
Gender		
M	75	
F	53	
Age		
<50	16	
≥50	112	
Other malignancies	83	59 (22–90)
Gender		
M	45	
F	38	
Age		
<50	25	
≥50	58	

Abbreviation: OBNTD-Other benign non-tumor diseases

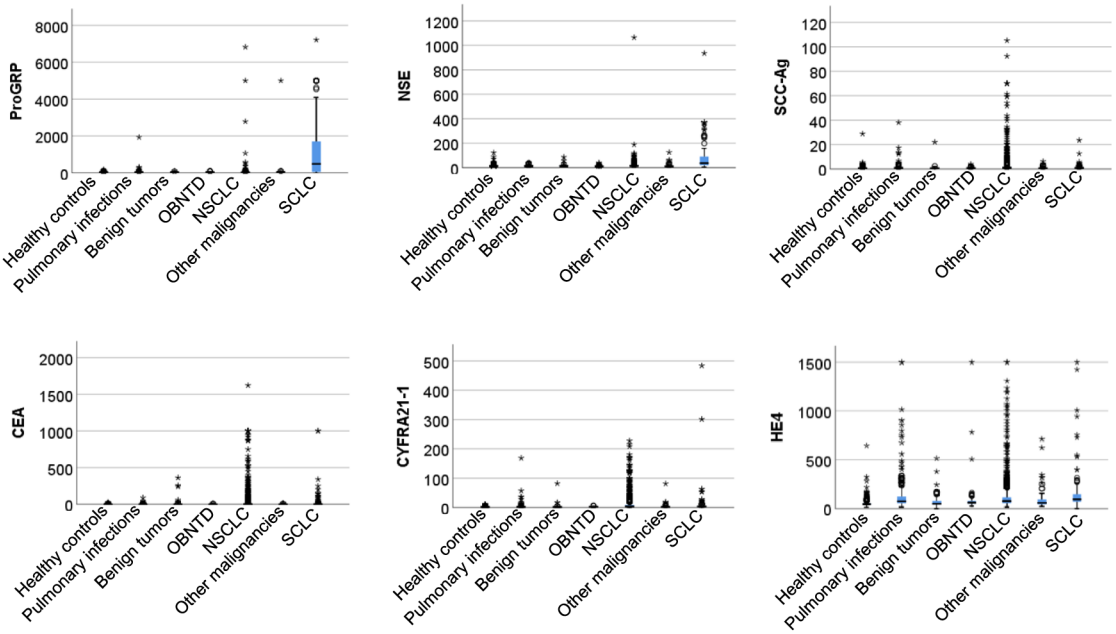


Figure 1. Comparison of lung cancer biomarkers among patient groups and healthy controls. The highest levels of ProGRP and NSE are seen in SCLC patients; the highest level of SCC-Ag is observed in NSCLC patients; the highest levels of CEA and CYFRA21-1 are seen in SCLC and NSCLC patients; while high levels of HE4 are seen in SCLC, followed by NSCLC and pulmonary infection patients.

and CYFRA21-1 levels between NSCLC and SCLC patients (p=0.172 and 0.125, respectively), further stratified analysis revealed that the highest CEA levels are observed in SCLC, however, there is no significant difference among adenocarcinoma (AD), SCC, and SCLC patient groups (Figure 2B). CYFRA21-1 shows high specificity to SCC as shown in Figure 2B. HE4 levels in SCLC patients are significantly higher than in NSCLC (p<0.0001) as seen in Figures 2A and 2B.

Association between serum biomarkers and clinical stages of SCLC and NSCLC (Mann Whitney U test). As shown in Figure 3, serum levels of all 6 biomarkers in advanced clinical stages (III+IV) in NSCLC are significantly higher than in early stages (I+II) (p=0.0017, p<0.001, p<0.0001, p<0.0001, p<0.0001, and p<0.0001, respectively). Extensive stage disease (ED) has higher ProGRP, NSE, and HE4 than limited stage disease (LD) in SCLC (p=0.0007, p<0.0001, and p=0.0020, respectively). However, results do not show significant difference in SCC-Ag, CEA, and CYFRA21-1 between LD and ED in SCLC (p=0.9250, p=0.4240, and p=0.0610, respectively).

Association between serum biomarkers and tumor size. Serum biomarker levels were compared with tumor size obtained by CT scan. Results indicated that tumor size larger than a sum of 10 mm in diameters had higher serum biomarker levels (p<0.05, for all), Figure 4.

Comparison of serum levels of 6 biomarkers between gender and between age groups (Mann-Whitney U test). Serum levels of NSE, SCC-Ag, CEA, CYFRA21-1, and HE4 in males were significantly higher than in females (p<0.0001 for all); there was no significant difference in ProGRP levels between males and females (p=0.3021). Serum levels of all biomarkers in ages ≥50 were significantly higher than in ages <50 (p<0.05 for all), Figure 5.

Diagnostic performance of 6 biomarkers on histological types of lung cancer (Receiver Operating Characteristic (ROC) curve). The diagnostic performance of a single biomarker or combinatory analysis on a specific histological type of lung cancer was analyzed. The results indicate that for the SCLC; NSE, HE4, and ProGRP present relatively high performance (AUC=0.8805, AUC=0.8545, and AUC=0.8340, respectively, Table 2), with NSE presenting the highest AUC, HE4 showing the highest sensitivity, and ProGRP having the highest specificity.

For SCC, CYFRA21-1, HE4, and SCC-Ag indicate relatively high performance (AUC=0.9103, AUC=0.8299, and AUC=0.7855, respectively, Table 2), with CYFRA21-1 showing the highest AUC and sensitivity, SCC-Ag having the highest specificity.

For adenocarcinoma histological type, the performance of all biomarkers was generally low, HE4, CYFRA21-1, and CEA have relatively high performance (AUC=0.7326, AUC=0.7264, and AUC=0.7108, respectively, Table 2), with HE4 presenting the highest AUC, CYFRA21-1 showing the highest sensitivity, and CEA having the highest specificity.

For NSCLC as a general histological type, the performance of all biomarkers was also low. CYFRA21-1, HE4, and CEA had relatively high performance (AUC=0.7867, AUC=0.7631, and AUC=0.7119, respectively, Table 2), with

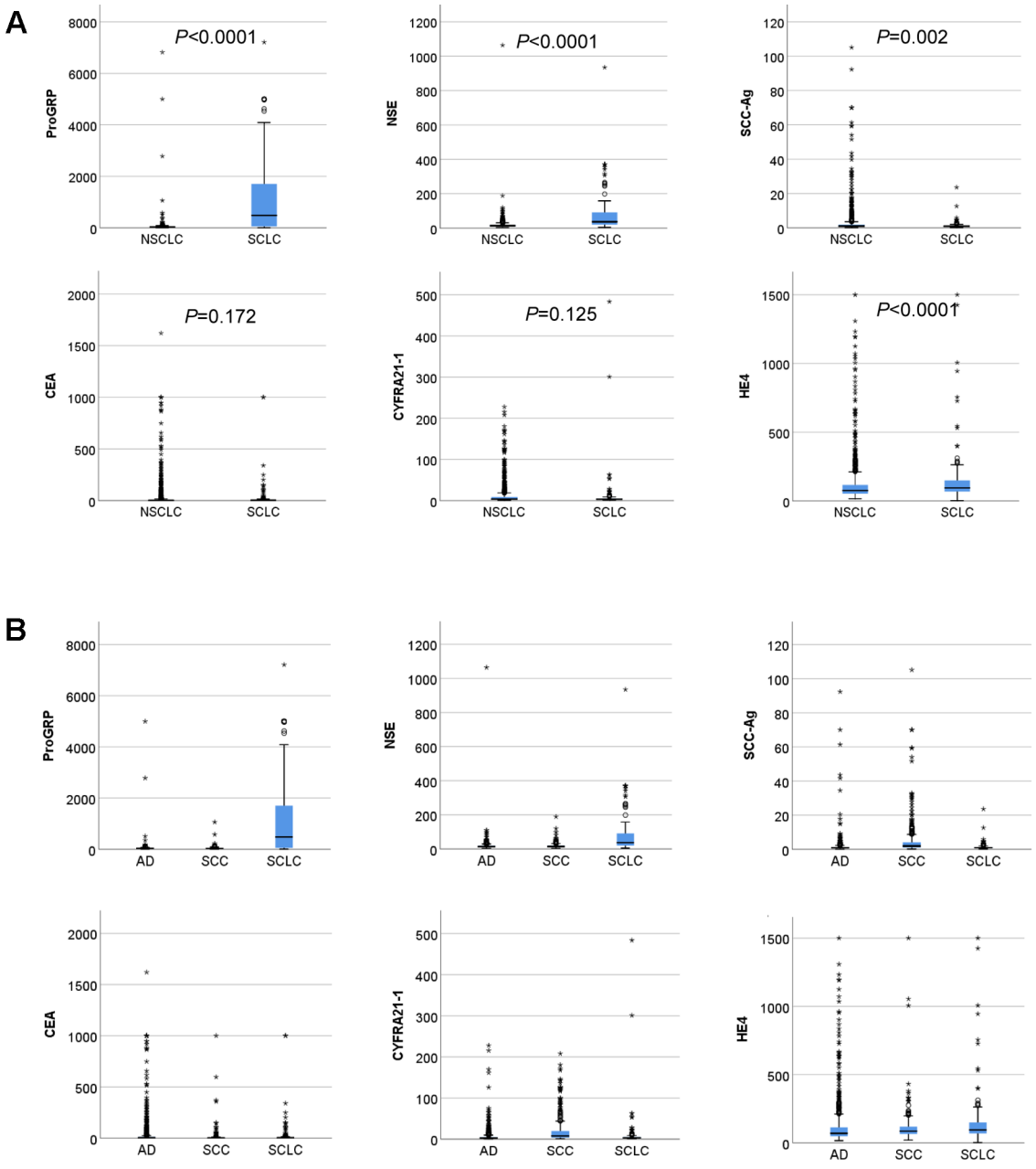


Figure 2. A, B) Serum ProGRP, NSE, SCC-Ag, CEA, CYFRA21-1, and HE4 levels in different histological types of lung cancer. ProGRP and NSE levels in SCLC are significantly higher than in NSCLC (p<0.0001); SCC-Ag level in NSCLC is significantly higher than in SCLC (p<0.0001) and is specific to SCC histological type; there is no significant difference in CEA and CYFRA21-1 levels between SCLC and NSCLC patients (p=0.172 and p=0.125, respectively), and CYFRA21-1 is also specific to SCC histological type; HE4 level in SCLC is significantly higher than in NSCLC (p<0.0001); HE4 is also specific to AD, secondary to SCLC, and then SCC.

CYFRA21-1 presenting the highest AUC, HE4 shows the highest sensitivity, and CEA having the highest specificity.

Different combinatory analyses of biomarkers were performed. For SCLC, combined analysis of ProGRP+NSE+HE4 gained the highest diagnostic performance (AUC=0.9282) (Table 2 and Suppl. Figure S2).

For SCC, a combined analysis of CYFRA21-1+SCC-Ag+CEA gained the highest diagnostic power (AUC=0.9240; Table 2 and Suppl. Figure S2).

For adenocarcinoma, a combinatory analysis of CYFRA21-1+CEA+HE4 enhanced the diagnostic performance (AUC=0.7653; Table 2 and Suppl. Figure S2).

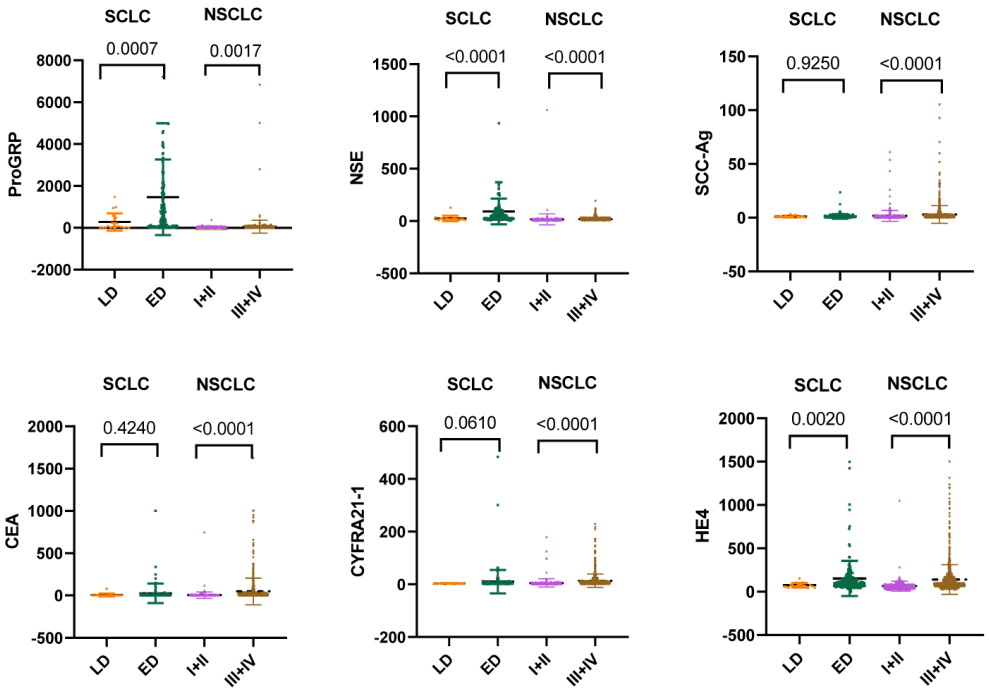


Figure 3. Association between serum biomarkers and clinical stage of SCLC and NSCLC. ProGRP, NSE, and HE4 levels are significantly higher in the advanced stage of tumors than in those with the early stage of both SCLC and NSCLC (p<0.05, for all); SCC-Ag, CEA, and CYFRA21-1 levels in the advanced stage of NSCLC are significantly higher than in the early stage (p<0.05, for all). However, there is no significant difference in these three markers' levels between clinical stages of SCLC (p>0.05).

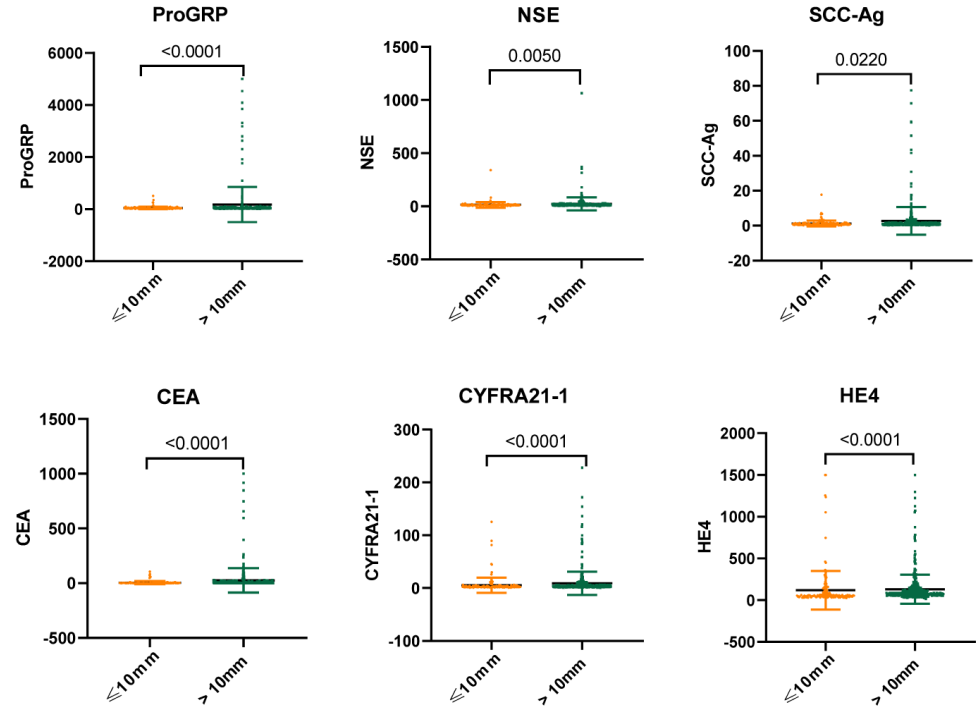


Figure 4. Association between serum biomarkers and tumor size. The levels of all 6 biomarkers show significantly higher in tumors >10 mm than in tumors <10 mm in diameters (p<0.05, for all).

Table 2. Diagnostic performance of 6 biomarkers on histological types of lung cancers.

		AUC	Youden's Index	Cut-off	Sensitivity	Specificity	PPV	NPV
SCLC	ProGRP	0.8340	0.6783	78.71	70.05	97.78	68.66	97.89
	NSE	0.8805	0.6531	19.40	76.14	89.16	31.45	98.13
	SCC-Ag	0.5463	0.1208	0.86	54.31	57.76	8.16	94.65
	CEA	0.7625	0.3889	1.63	82.74	56.74	11.68	97.87
	CYFRA21-1	0.8065	0.4724	2.37	76.14	71.10	15.42	97.67
	HE4	0.8545	0.6229	62.55	83.76	78.53	21.54	98.56
	ProGRP+NSE	0.9272	0.8027	0.07	82.74	97.50	69.96	98.77
	ProGRP+NSE+HE4	0.9282	0.8036	0.05	83.76	96.57	63.22	98.83
SCC	ProGRP	0.5893	0.2353	23.31	34.79	88.73	9.78	68.93
	NSE	0.5881	0.1773	15.63	42.34	75.39	20.14	89.88
	SCC-Ag	0.7855	0.5015	1.48	59.12	91.02	48.80	93.80
	CEA	0.7164	0.2999	1.96	63.50	66.49	21.64	92.50
	CYFRA21-1	0.9103	0.7015	3.25	81.02	89.13	51.88	96.92
	HE4	0.8299	0.5621	63.68	76.64	79.57	35.51	95.86
	CYFRA21-1+SCC-Ag	0.9238	0.7300	0.10	81.51	91.45	58.36	97.11
	CYFRA21-1+SCC-Ag+CEA	0.9240	0.7287	0.10	81.02	91.81	59.25	97.05
AD	ProGRP	0.5588	0.1174	31.12	41.53	70.21	20.69	69.71
	NSE	0.555	0.1163	16.42	32.27	79.36	32.79	78.93
	SCC-Ag	0.5296	0.0890	1.33	21.74	87.16	33.99	78.02
	CEA	0.7108	0.3399	3.48	42.79	91.20	59.84	83.58
	CYFRA21-1	0.7264	0.3203	2.98	66.34	85.69	49.57	83.61
	HE4	0.7326	0.3651	56.12	64.99	71.52	41.61	86.72
	CYFRA21-1+ CEA	0.7641	0.4087	0.23	49.89	90.38	62.11	85.08
	CYFRA21-1+CEA+HE4	0.7653	0.4094	0.02	49.89	90.45	62.29	85.09
NSCLC	ProGRP	0.5673	0.1447	23.9	26.60	87.88	28.24	49.93
	NSE	0.567	0.1378	15.88	36.78	77.00	42.89	72.14
	SCC-Ag	0.6122	0.2158	1.47	30.70	90.88	60.53	73.59
	CEA	0.7119	0.3163	3.48	40.43	91.20	67.98	76.47
	CYFRA21-1	0.7867	0.4419	2.77	62.46	81.72	61.43	82.19
	HE4	0.7631	0.4263	60.37	66.03	76.60	57.02	82.73
	CYFRA21-1+CEA	0.8109	0.4999	0.30	60.26	87.72	73.36	79.74
	CYFRA21-1+CEA+HE4	0.8110	0.4960	0.27	62.77	86.80	69.12	83.20

Abbreviations: AD-adenocarcinoma; AUC-Area Under Curve; PPV-positive predictive value; NPV-negative predictive value

For NSCLC as a general histological type, the highest diagnostic performance was obtained by a 3-marker combination of CYFRA21-1+CEA+HE4, the AUC, sensitivity, and specificity are 0.8110, 62.77, and 86.80, respectively (Table 2 and Suppl. Figure S2).

More diagnostic performance-related parameters can be seen in Table 2.

Discussion

The results from this study provide further insights into the value of 6 biomarkers in lung cancer diagnosis including HE4.

The role of serum ProGRP, NSE, SCC-Ag, CEA, CYFRA21-1, and HE4 levels in characterizing lung cancer. Our results showed that all 6 biomarkers had the capability of characterizing lung cancer (SCLC or NSCLC, or both). However, HE4 was also elevated in pulmonary infections. It was also slightly increased in other diseases (Figure 1 and Suppl. Table S2). A previous study revealed that HE4 was elevated in pulmonary tuberculosis, suggesting a potential clinical significance [20]. Other reports mentioned that HE4 was increased in cystic pulmonary fibrosis [21, 22]. From this context, caution should be used when interpreting an elevated serum level of HE4 in clinics.

Comparison of lung cancer biomarkers among histological types. As seen in Figure 1 and Figures 2a and 2b, ProGRP and NSE levels in SCLC are significantly higher than in other histological types. This is consistent with other reports previously [23, 24]. Because it is highly specific to SCLC histological type, NSE could be used as an indicator of histological transformation during lung cancer tyrosine kinase inhibitor treatment [25].

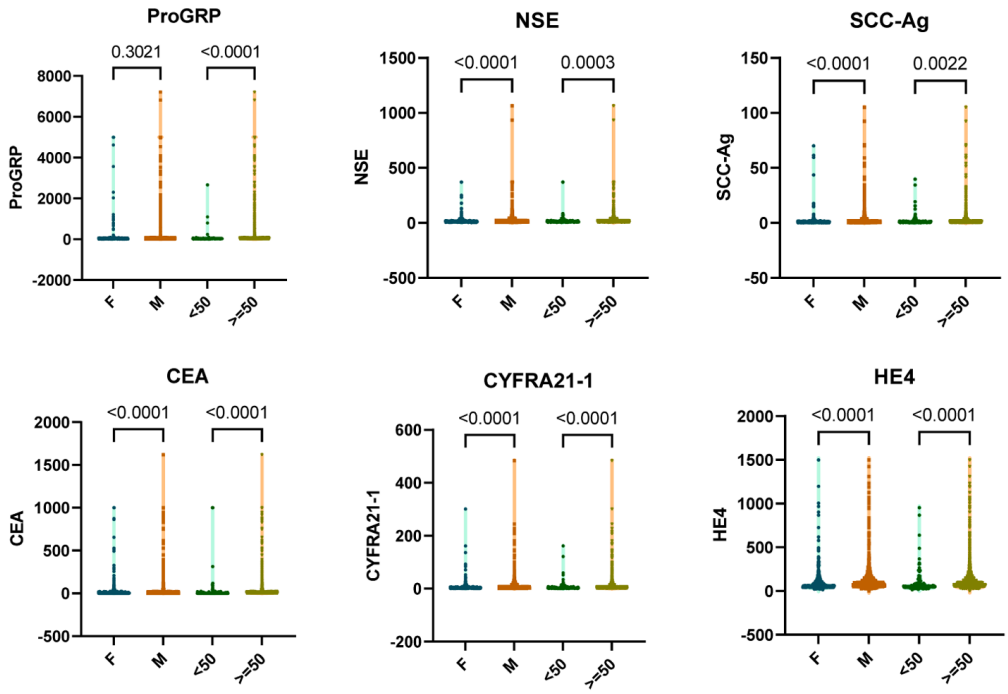


Figure 5. Comparison of lung cancer biomarkers among genders and age groups. NSE, SCC-Ag, CEA, CYFRA21-1, and HE4 levels in male patients are significantly higher than in females (p<0.0001 for all); there is no significant difference in ProGRP levels between males and females (p=0.3021). Serum levels of all biomarkers in patients aged >50 are significantly higher than in ages <50 (p<0.05 for all).

It is known that SCC-Ag increased in SCC of different origins. However, using SCC-Ag as a biomarker for NSCLC, specifically for SCC, is still debatable because of its low sensitivity. Our results showed that the levels of SCC-Ag were significantly elevated in NSCLC including SCC histological type (Figures 1, 2A, 2B). These results are similar to other reports [26–28].

CEA is considered a biomarker for SCLC and adenocarcinoma (AD) of the lung [29], and serum CEA is elevated in many types of cancers mostly in AD [30, 31]. CEA is also increased in other types of non-cancerous diseases or other conditions [11, 32]. Our results showed that high levels of CEA were observed in both NSCLC (which includes AD and SCC) and SCLC (Figures 1, 2A, 2B). Elevation of serum CEA was also found in patients with SCC of the anus, and it was associated with recurrence in some patients [33]. Sadeghi et al. found that CEA was a useful biomarker in characterizing SCC from basal cell carcinoma [34]. However, one study mentioned that although CEA was increased in anal cancer, but it did not have value in survival prediction, thus it is not a clinical biomarker for SCC [35]. Therefore, CEA is more useful in characterizing AD and SCLC.

Our results show that CYFRA21-1 is significantly increased in NSCLC, basically in SCC (Figures 1, 2A, 2B), which is consistent with previous reports [36–40].

High levels of HE4 were seen in SCLC followed by NSCLC (including AD and SCC), then pulmonary infection. Wang et al. found that high serum levels of HE4 were consistent with the high histological expression level of HE4 in SCLC histological type of lung cancer [41] and was also observed in serum [42]. Iwahori et al. reported that a high level of HE4 was found in both NSCLC and SCLC lung cancer patients [43]. One study reported that HE4 did not show a significant difference between different histological subgroups of lung cancer. However, a significant correlation was found between HE4 values and the tumor size [18]. Contradictorily, another study showed that adenocarcinoma had a higher HE4 expression level, while SCLC had the lowest expression of the marker [44]. The contradictory results could be attributed to study design, such as patient enrollment and impact of treatment, as well as the quality of testing. More studies are needed to verify the histological expression pattern of this protein in lung cancer.

Association between lung cancer biomarkers and clinical stages and tumor size. We found that the levels of all biomarkers were significantly higher in the advanced stage than in the early stage of NSCLC. ProGRP, NSE, and HE4 in extensive stage disease (ED) were significantly higher than in limited stage disease (LD) in SCLE. No significant difference was seen in SCC-Ag, CEA, and CYFRA21-1 levels between LD and ED stages in SCLC patients (Figure 3). Previous reports also mentioned that elevated lung cancer biomarkers were associated with nodal involvement and distant metastasis [45–47].

In addition, higher serum biomarkers levels were seen in larger tumor sizes determined by CT scans (Figure 4).

Serum levels of 6 biomarkers among gender and age groups. Apart from ProGRP, serum levels of NSE, SCC-Ag, CEA, CYFRA21-1, and HE4 were higher in male patients than in females. All biomarkers observed in this study show higher levels in patients aged 50 or older than under 50. This phenomenon was similar to what we had observed in our previous study in apparently healthy individuals [48]. These results suggest that gender and age should be considered when making a clinical evaluation; thus, a baseline of an individual biomarker for gender and age should be established in clinical practice. In addition, the patient's baseline of biomarkers and timely monitoring, when necessary, should be considered in clinical practice.

Diagnostic performance of ProGRP, NSE, SCC-Ag, CEA, CYFRA21-1, and HE4 on histological types of lung cancers. Generally, CEA is considered a biomarker for lung adenocarcinoma, and SCC-Ag is considered a biomarker for SCC. CYFRA21-1 is considered a biomarker for NSCLC, while ProGRP and NSE are considered biomarkers for SCLC. Nevertheless, the study revealed that a high level of NSE was also found in NSCLC patients, reflecting the response to epidermal growth factor receptor tyrosine kinase inhibitor treatment; and elevation of serum NSE was associated with shorter survival of the NSCLC patients [49–51]. Our results indicated that NSE levels were moderately increased in NSCLC, secondary to SCLC; and the diagnostic performance of NSE for SCLC was better than for NSCLC, it was consistent with serum levels (Table 2, Suppl. Table S2).

One study found that CYFRA21-1 had a greater sensitivity for recurrent stages of SCLC [52]. Our results revealed that SCLC patient group had the highest CYFRA21-1 levels, however, the diagnostic performance of CYFRA21-1 alone was not as good as other biomarkers (Suppl. Table S2 and Table 2).

Our results indicated that SCLC had the highest HE4 level, followed by NSCLC and pulmonary infections (Suppl. Table S2). When looking at the diagnostic performance of 6 biomarkers individually on histological types, NSE, HE4, and ProGRP showed relatively high AUC, sensitivity, and specificity for SCLC. These results are similar to other reports on ProGRP [53–55].

It is known that combinatory analysis of tumor markers can enhance diagnostic power. The key is to find out the best combination for clinical use. Our results showed that the three-biomarkers combination as ProGRP+NSE+HE4 gained the highest diagnostic performance for SCLC (Table 2, Suppl. Figure S2).

CYFRA21-1, HE4, and SCC-Ag had a relatively high diagnostic performance on SCC; the three-biomarkers combination as CYFRA21-1+SCC-Ag+CEA showed the highest diagnostic power (Table 2, Suppl. Figure S2).

HE4, CYFRA21-1, and CEA had a relatively high performance on adenocarcinoma; the three-markers combination as CYFRA21-1+CEA+HE4 indicated the highest diagnostic power as seen in Table 2 and Suppl. Figure S2.

Taking NSCLC as a collective histological group, the three-markers combination CYFRA21-1+CEA+HE4 showed the highest diagnostic performance (Table 2, Suppl. Figure S2).

To our surprise, the diagnostic performance of all 6 biomarkers for adenocarcinoma was generally low. The best single biomarker for adenocarcinoma in terms of AUC was HE4 (AUC 0.7326), and the best performance of a three-markers combination CYFRA21-1+CEA+HE4 displayed only 0.7651 in AUC (Table 2, Suppl. Figure S2). Considering that adenocarcinoma of the lung accounts for about 30–40% of all lung cancers, new biomarkers with more sensitive to this form of lung cancer are needed.

In conclusion, serum ProGRP, NSE, SCC-Ag, CEA, CYFRA21-1, and HE4 were able to characterize lung cancer from other diseases and healthy individuals with NSE and ProGRP were two ideal biomarkers for characterizing SCLC, while SCC-Ag was a fair marker for NSCLC, specifically for SCC diagnosis. Elevation of serum CEA or CYFRA21-1 could be suggestive of SCLC and NSCLC histotype. HE4 showed high specificity to SCLC, and it increased the diagnostic sensitivity when combined with other biomarkers. Thus, HE4 could be a potential serum biomarker for lung cancer diagnosis. New biomarkers with more sensitive to adenocarcinoma of the lung are needed.

Ethical statement

The authors are responsible for the accuracy of the study results. This study was approved by the Institutional Review Board (Medical Ethics Committee of Renmin Hospital of Wuhan University, the First Affiliated Hospital of University of Science and Technology of China, Qilu Hospital of Shandong University, Shaanxi Provincial People's Hospital, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Suzhou Municipal Hospital, Henan Provincial People's Hospital, Nanjing First Hospital of Nanjing Medical University, and Xiangya Hospital of Central South University. Patients' informed consent was waivered by the participating hospital's ethics committee.

Supplementary information is available in the online version of the paper.

Acknowledgments: We would like to thank Dr. Liejun Jiang, Dr. Zhijiang Mo of the People's Hospital of Guangxi Zhuang Autonomous Region, and Dr. Zhiju Li of Southern Medical University for performing the statistical analysis and tedious discussion.

References

[1] SIEGEL RL, MILLER KD, JEMAL A. Cancer statistics, 2020. CA Cancer J Clin 2020; 70: 7–30. https://doi.org/10.3322/caac.21590

[2] TRIPHURIDET N, VIDHYARKORN S, WORAKITSITISATORN A, SRICHARUNRAT T, TEERAYATHANAKUL N et al. Screening values of carcinoembryonic antigen and cytokeratin 19 fragment for lung cancer in combination with low-dose computed tomography in high-risk populations: Initial and 2-year screening outcomes. Lung Cancer 2018; 122: 243–248. https://doi.org/10.1016/j.lungcan.2018.05.012

[3] GRUNNET M, SORENSEN JB. Carcinoembryonic antigen (CEA) as tumor marker in lung cancer. Lung Cancer 2012; 76: 138–143. https://doi.org/10.1016/j.lungcan.2011.11.012

[4] YANG DW, ZHANG Y, HONG QY, HU J, LI C et al. Role of a serum-based biomarker panel in the early diagnosis of lung cancer for a cohort of high-risk patients. Cancer 2015; 121: 3113–3121. https://doi.org/10.1002/cncr.29551

[5] DING PN, BECKER TM, BRAY VJ, CHUA W, MA YF et al. The predictive and prognostic significance of liquid biopsy in advanced epidermal growth factor receptor-mutated non-small cell lung cancer: A prospective study. Lung Cancer 2019; 134: 187–193. https://doi.org/10.1016/j.lungcan.2019.06.021

[6] MORITA S, SUDA T, ODA C, KOBAYASHI M, HOSHI T et al. The Value of 18F-FDG PET in the Diagnosis of Intertrabecular Vertebral Metastasis in a Small Cell Lung Cancer Patient with a High Serum CEA Level. Intern Med 2019; 58: 415–418. https://doi.org/10.2169/internalmedicine.1394-18

[7] MULEY T, ZHANG X, HOLDENRIEDER S, KORSE CM, ZHI XY et al. A continuous responder algorithm to optimize clinical management of small-cell lung cancer with progastrin-releasing peptide as a simple blood test. Tumour Biol 2020; 42: 1010428320958603. https://doi.org/10.1177/1010428320958603

[8] HONDA Y, KATAGIRI H, TAKAHASHI M, MURATA H, WASA J et al. Pro-gastrin-releasing peptide as a marker for the Ewing sarcoma family of tumors. Int J Clin Oncol 2019; 24: 1468–1478. https://doi.org/10.1007/s10147-019-01492-0

[9] SARGIN G, KÖSE R, ŞENTÜRK T. Tumor-Associated Antigens in Rheumatoid Arthritis Interstitial Lung Disease or Malignancy? Arch Rheumatol 2018; 33: 431–437. https://doi.org/10.5606/ArchRheumatol.2018.6691

[10] HAO C, ZHANG G, ZHANG L. Serum CEA levels in 49 different types of cancer and noncancer diseases. Prog Mol Biol Transl Sci 2019; 162: 213–227. https://doi.org/10.1016/bs.pmbts.2018.12.011

[11] KOMATSU H, IZUMI N, TSUKIOKA T, TODA M, HARA K. Elevation of Serum Carcinoembryonic Antigen Concentration Caused by Everolimus-Induced Lung Injury: A Case Report. Ann Thorac Cardiovasc Surg 2018; 24: 151–153. https://doi.org/10.5761/atcs.cr.17-00092

[12] NOMOTO H, MIYOSHI H, SEKIZAKI T, ATSUMI T. Comment on "Elevation of Serum Carcinoembryonic Antigen Concentration Caused by Everolimus-Induced Lung Injury: A Case Report". Ann Thorac Cardiovasc Surg 2018; 24: 165–166. https://doi.org/10.5761/atcs.lte.18-00018

[13] GU T, WEN Z, XU S, HUA H, ZHANG Z et al. Decreased levels of circulating sex hormones as a biomarker of lung cancer in male patients with solitary pulmonary nodules. Afr Health Sci 2014; 14: 356–363. https://doi.org/10.4314/ahs.v14i2.10

[14] ESCUDERO JM, AUGE JM, FILELLA X, TORNE A, PAHISA J et al. Comparison of serum human epididymis protein 4 with cancer antigen 125 as a tumor marker in patients with malignant and nonmalignant diseases. Clin Chem 2011; 57: 1534–1544. https://doi.org/10.1373/clinchem.2010.157073

[15] LIU W, YANG J, CHI PD, ZHENG X, DAI SQ et al. Evaluating the clinical significance of serum HE4 levels in lung cancer and pulmonary tuberculosis. Int J Tuberc Lung Dis 2013; 17: 1346–1353. https://doi.org/10.5588/ijtld.13.0058

[16] YAN L, HU ZD. Diagnostic accuracy of human epididymis secretory protein 4 for lung cancer: a systematic review and meta-analysis. J Thorac Dis 2019; 11: 2737–2744. https://doi.org/10.21037/jtd.2019.06.72

[17] WANG Y, WANG Z, DING Y, SUN F, DING X. The Application Value of Serum HE4 in the Diagnosis of Lung Cancer. Asian Pac J Cancer Prev 2019; 20: 2405–2407. https://doi.org/10.31557/APJCP.2019.20.8.2405

[18] NAGY B JR, BHATTOA HP, STEIBER Z, CSOBÁN M, SZILASI M et al. Serum human epididymis protein 4 (HE4) as a tumor marker in men with lung cancer. Clin Chem Lab Med 2014; 52: 1639–1648. https://doi.org/10.1515/cclm-2014-0041

[19] CLSI. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. 3rd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.

[20] LI L, YAO Y, LIANG J, ZHAN X, WANG F et al. Serum human epididymis protein 4 concentrations are associated with severity of patients with pulmonary tuberculosis. Clin Chim Acta 2020; 502: 255–260. https://doi.org/10.1016/j.cca.2019.11.009

[21] WANG J, ZHAO H, XU F, ZHANG P, ZHENG Y et al. Human epididymis protein 4 (HE4) protects against cystic pulmonary fibrosis associated-inflammation through inhibition of NF-κB and MAPK singnaling. Genes Genomics 2019; 41: 1045–1053. https://doi.org/10.1007/s13258-019-00836-4

[22] NAGY B JR, NAGY B, FILA L, CLARKE LA, GÖNCZY F et al. Human Epididymis Protein 4: A Novel Serum Inflammatory Biomarker in Cystic Fibrosis. Chest 2016; 150: 661–672. https://doi.org/10.1016/j.chest.2016.04.006

[23] DU J, LI Y, WANG L, ZHOU Y, SHEN Y et al. Selective application of neuroendocrine markers in the diagnosis and treatment of small cell lung cancer. Clin Chim Acta 2020; 509: 295–303. https://doi.org/10.1016/j.cca.2020.06.037

[24] DONG A, ZHANG J, CHEN X, REN X, ZHANG X. Diagnostic value of ProGRP for small cell lung cancer in different stages. J Thorac Dis 2019; 11: 1182–1189. https://doi.org/10.21037/jtd.2019.04.29

[25] CHEN S, HE Y, LIU J, CHEN X, YU J et al. Third-Generation TKI Resistance Due to SCLC Transformation: A Case Report and Brief Review. Onco Targets Ther 2019; 12: 11305–11311. https://doi.org/10.2147/OTT.S228301

[26] MORO D, VILLEMAIN D, VUILLEZ JP, DELORD CA, BRAMBILLA C. CEA, CYFRA21-1 and SCC in non-small cell lung cancer. Lung Cancer 1995; 13: 169–176. https://doi.org/10.1016/0169-5002(95)00485-8

[27] REINMUTH N, BRANDT B, SEMIK M, KUNZE WP, ACHATZY R et al. Prognostic impact of Cyfra21-1 and other serum markers in completely resected non-small cell lung cancer. Lung Cancer 2002; 36: 265–270. https://doi.org/10.1016/s0169-5002(02)00009-0

[28] KAGOHASHI K, SATOH H, ISHIKAWA H, OHTSUKA M, SEKIZAWA K. A re-evaluation of squamous cell carcinoma antigen (SCC) as a serum marker for non-small cell lung cancer. Med Oncol 2008; 25: 187–189. https://doi.org/10.1007/s12032-007-9021-3

[29] MEHTA A, PARKASH A, BHATIA M. Cross-Sectional Study to Establish the Utility of Serum Tumor Markers in the Diagnosis of Lung Cancer. Asian Pac J Cancer Prev 2021; 22: 2569–2576. https://doi.org/10.31557/APJCP.2021.22.8.2569

[30] LUGAT A, HULO P, ANSQUER C, TOUCHEFEU Y, MIRALLIÉ E et al. Carcinoembryonic Antigen Increase in a Patient with Colon Cancer Who Have Achieved Complete Remission and Negative 18F-FDG PET/CT: Don't Forget the Thyroid! Curr Oncol 2021; 28: 2987–2992. https://doi.org/10.3390/curroncol28040261

[31] GOU Q, FU S, XIE Y, ZHANG M, SHEN Y. Treatment and Survival Patterns of Primary Adenosquamous Carcinoma of the Liver: A Retrospective Analysis. Front Oncol 2021; 11: 621594. https://doi.org/10.3389/fonc.2021.621594

[32] SUN Y, GE YL, LI LQ, LIU Y, LU Y et al. Elevated carcinoembryonic antigen and bronchial obstruction caused by a rotten vegetable leaf mimic lung cancer: A case report. J Clin Lab Anal 2021; 35: e23579. https://doi.org/10.1002/jcla.23579

[33] RAAB GT, O'NEIL DS, KIRAN RP, FEINGOLD DL, LEE-KONG SA et al. Elevation of Serum CEA in Patients with Squamous Cell Carcinoma of the Anus. Cancer Invest 2019; 37: 288–292. https://doi.org/10.1080/07357907.2019.1636388

[34] RAMEZANI M, MOHAMADZAHERI E, KHAZAEI S, NAJAFI F, VAISI-RAYGANI A et al. Comparison of EMA, CEA, CD10 and Bcl-2 Biomarkers by Immunohistochemistry in Squamous Cell Carcinoma and Basal Cell Carcinoma of the Skin. Asian Pac J Cancer Prev 2016; 17: 1379–1383. https://doi.org/10.7314/apjcp.2016.17.3.1379

[35] HESTER R, ADVANI S, RASHID A, HOLLIDAY E, MESSICK C et al. CEA as a blood-based biomarker in anal cancer. Oncotarget 2021; 12: 1037–1045. https://doi.org/10.18632/oncotarget.27959

[36] WIESKOPF B, DEMANGEAT C, PUROHIT A, STENGER R, GRIES P et al. Cyfra 21-1 as a biologic marker of non-small cell lung cancer. Evaluation of sensitivity, specificity, and prognostic role. Chest 1995; 108: 163–169. https://doi.org/10.1378/chest.108.1.163

[37] SONE K, OGURI T, NAKAO M, KAGAWA Y, KUROWAKA R et al. CYFRA 21-1 as a Predictive Marker for Non-small Cell Lung Cancer Treated with Pemetrexed-based Chemotherapy. Anticancer Res 2017; 37: 935–939. https://doi.org/10.21873/anticanres.11402

[38] RASTEL D, RAMAIOLI A, CORNILLIE F, THIRION B. CYFRA 21-1, a sensitive and specific new tumour marker for squamous cell lung cancer. Report of the first European multicentre evaluation. CYFRA 21-1 Multicentre Study Group. Eur J Cancer 1994; 30A: 601–606. https://doi.org/10.1016/0959-8049(94)90528-2

[39] TAS F, AYDINER A, TOPUZ E, YASASEVER V, KARADENIZ A et al. Utility of the serum tumor markers: CYFRA 21.1, carcinoembryonic antigen (CEA), and squamous cell carcinoma antigen (SCC) in squamous cell lung cancer. J Exp Clin Cancer Res 2000; 19: 477–481.

[40] YAMAMOTO K, OKA M, HAYASHI H, TANGOKU A, GONDO T et al. CYFRA 21-1 is a useful marker for esophageal squamous cell carcinoma. Cancer 1997; 79: 1647–1655.

[41] WANG X, FAN Y, WANG J, WANG H, LIU W. Evaluating the expression and diagnostic value of human epididymis protein 4 (HE4) in small cell lung cancer. Tumour Biol 2014; 35: 6847–6853. https://doi.org/10.1007/s13277-014-1943-8

[42] WOJCIK E, TARAPACZ J, RYCHLIK U, STASIK Z, SASKORCZYNSKA B et al. Human Epididymis Protein 4 (HE4) in Patients with Small-Cell Lung Cancer. Clin Lab 2016; 62: 1625–1632. https://doi.org/10.7754/Clin.Lab.2016.151212

[43] IWAHORI K, SUZUKI H, KISHI Y, FUJII Y, UEHARA R et al. Serum HE4 as a diagnostic and prognostic marker for lung cancer. Tumour Biol 2012; 33: 1141–1149. https://doi.org/10.1007/s13277-012-0356-9

[44] CELIK B, BULUT T. Human epididymis protein 4 may not be a reliable screening biomarker for detecting lung carcinoma patients. Biomed Rep 2017; 7: 297–300. https://doi.org/10.3892/br.2017.971

[45] ISGRÒ MA, BOTTONI P, SCATENA R. Neuron-Specific Enolase as a Biomarker: Biochemical and Clinical Aspects. Adv Exp Med Biol 2015; 867: 125–143. https://doi.org/10.1007/978-94-017-7215-0_9

[46] NASRALLA A, LEE J, DANG J, TURNER S. Elevated preoperative CEA is associated with subclinical nodal involvement and worse survival in stage I non-small cell lung cancer: a systematic review and meta-analysis. J Cardiothorac Surg 2020; 15: 318. https://doi.org/10.1186/s13019-020-01353-2

[47] GUO D, JING W, ZHU H, LI M, ZOU B et al. Clinical value of carcinoembryonic antigen for predicting the incidence of brain metastases and survival in small cell lung cancer patients treated with prophylactic cranial irradiation. Cancer Manag Res 2018; 10: 3199–3205. https://doi.org/10.2147/CMAR.S175043

[48] LI Y, LI M, ZHANG Y, ZHOU J, JIANG L et al. Age-stratified and gender-specific reference intervals of six tumor markers panel of lung cancer: A geographic-based multicenter study in China. J Clin Lab Anal 2021; 35: e23816. https://doi.org/10.1002/jcla.23816

[49] YAN P, HAN Y, TONG A, LIU J, WANG X et al. Prognostic value of neuron-specific enolase in patients with advanced and metastatic non-neuroendocrine non-small cell lung cancer. Biosci Rep 2021; 41: BSR20210866. https://doi.org/10.1042/BSR20210866

[50] TRAVIS W, BRAMBILLA E, MÜLLER-HERMELING H, HARRIS CC (EDS.). The concept of pulmonary neuroendocrine tumours. In: Pathology and genetics of tumours of the lung, pleura, thymus and heart. IARC Press, Lyon 2004, p. 353. ISBN 92 832 2418 3

[51] SUH KJ, KEAM B, KIM M, PARK YS, KIM TM et al. Serum Neuron-Specific Enolase Levels Predict the Efficacy of First-Line Epidermal Growth Factor Receptor (EGFR) Tyrosine Kinase Inhibitors in Patients with Non-Small Cell Lung Cancer Harboring EGFR Mutations. Clin Lung Cancer 2016; 17: 245–252.e1. https://doi.org/10.1016/j.cllc.2015.11.012

[52] CEDRÉS S, NUÑEZ I, LONGO M, MARTINEZ P, CHECA E et al. Serum tumor markers CEA, CYFRA21-1, and CA-125 are associated with worse prognosis in advanced non-small-cell lung cancer (NSCLC). Clin Lung Cancer 2011; 12: 172–179. https://doi.org/10.1016/j.cllc.2011.03.019

[53] MAURO C, PASSERINI R, SPAGGIARI L, GALETTA D, RADICE D et al. New and old biomarkers in the differential diagnosis of lung cancer: Pro-gastrin-releasing peptide in comparison with neuron-specific enolase, carcinoembryonic antigen, and CYFRA 21-1. Int J Biol Markers 2019; 34: 163–167. https://doi.org/10.1177/1724600819834235

[54] KORKMAZ ET, KOKSAL D, AKSU F, DIKMEN ZG, ICEN D et al. Triple test with tumor markers CYFRA 21.1, HE4, and ProGRP might contribute to diagnosis and subtyping of lung cancer. Clin Biochem 2018; 58: 15–19. https://doi.org/10.1016/j.clinbiochem.2018.05.001

[55] KUDO K, OHYANAGI F, HORIIKE A, MIYAUCHI E, YANAGITANI N et al. Clinicopathological findings of non-small-cell lung cancer with high serum progastrin-releasing peptide concentrations. Lung Cancer 2011; 74: 401–404. https://doi.org/10.1016/j.lungcan.2011.03.019

https://doi.org/10.4149/neo_2022_220207N144

New insights into the diagnostic characteristics and clinical application of serum biomarkers for lung cancer, and human epididymis protein 4 as a new biomarker?

Ming LI[1,#], Yi ZHANG[2,#], Li JIANG[3], Yan LI[4], Gang LI[5], Jianping ZHOU[6], Chen YANG[7], Xinhui LI[8], Wei QU[9], Yong CHEN[10], Qing CHEN[10], Shukui WANG[11,*], Jinliang XING[12,*], Huayi HUANG[10,13,*]

Supplementary Information

Supplementary Table S1. Detailed patient demographics of non-lung cancers.

Groups	Diagnosis	n
Benign tumors		84
	Colon Polyps	15
	Ovarian cysts	7
	Uterine leiomyoma	44
	Benign thyroid nodules	1
	Mediastinum cyst	1
	Hamartoma	1
	Pulmonary angioma	1
Pulmonary infection		388
	COPD	31
	Pneumonia	212
	Pulmonary cavity	1
	Bronchiectasis	18
	Bronchial asthma	10
	Pulmonary fibrosis	10
	Pulmonary tuberculosis	50
	Pulmonary benign nodule	49
	Inflammatory pseudotumor of the lung	8
	Tuberculous pleuritis	6
	Pleuritis (non-TB)	2
OBNTD		128
	Hypertension	8
	Coronary heart disease	9
	Cerebral hemorrhage	3
	Posterior circulation ischemia	28
	Cerebral infarction	31
	Parkinson's disease	5
	Neuralgia	1
	Diabetes	4
	Hyperthyroidism	1
	Thyreoitis	1
	Osteoporosis	2
	Connective tissue disease	3
	Sjogren's syndrome	1
OBNTD *cont.*	Rheumatoid arthritis	1
	Non-infective fever	2
	Esophageal stenosis	1
	Enteritis	7
	Cholecystitis	1
	Varix of lower limb	3
	Cerebellar ataxia	1
	Brucellosis	1
	Superior mesenteric artery dissection	1
	Grand mal epilepsy	1
	Septic shock	1
	Fever of unknown origin	1
	Amyasthenia	1
	Feeble	1
	Infective fever	2
	Frozen shoulder	1
	Sleep-disorder	1
	Lumbago	1
	Nocturnal hypoxemia	1
	Pulmonary embolism	2
Other malignancies		83
	Breast cancer	8
	Pancreatic cancer	8
	Thyroid carcinoma	6
	Malignant neoplasm of endocrine gland	8
	Hematological malignances	8
	Thymic tumor	3
	Epithelioid sarcoma	3
	Esophageal cancer	3
	Gastric cancer	19
	Colorectal cancer	9
	Hepatoma	1
	Cervical cancer	3
	Thyroid carcinoma	4

Abbreviations: COPD-Chronic obstructive pulmonary disease; TB-tuberculosis; OBNTD-other benign non-tumor diseases

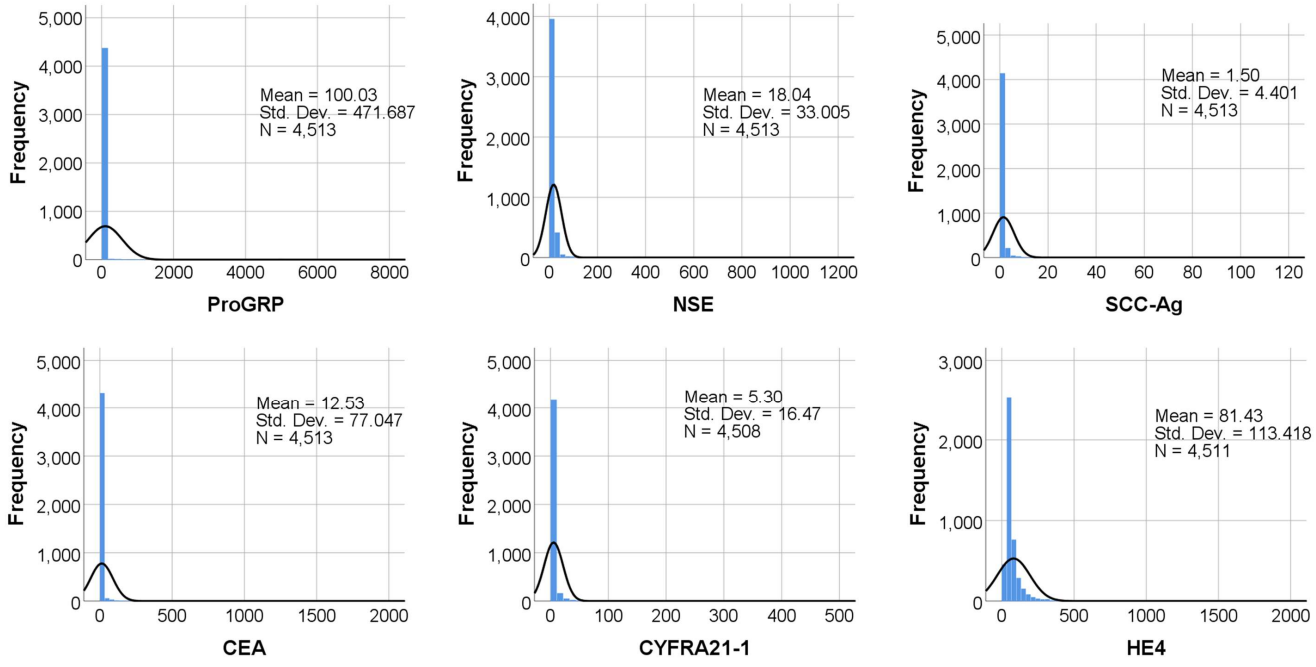
Supplementary Table S2. Statistical results of Figure 1 (sub-classification based on serum biomarkers concentrations and comparisons of the sub-sets).

Homogeneous subsets based on patient groups (ProGRP)					
		Subset			
		1	2	3	
Sample[a]	NSCLC	1932.536			
	Healthy controls		2239.118		
	OBNTD		2269.441		
	Benign tumors		2324.857		
	Pulmonary infections		2365.760		
	Other malignancies		2400.584		
	SCLC			3673.259	
Test Statistic		b	5.063	b	
Sig. (2-sided test)			0.281		
Adjusted Sig. (2-sided test)			0.370		
Homogeneous subsets based on patient groups (NSE)					
		Subset			
		1	2	3	4
Sample[a]	OBNTD	1413.695			
	Other malignancies	1890.458	1890.458		
	Healthy controls		2086.888		
	Benign tumors		2105.321		
	Pulmonary infections		2172.983		
	NSCLC			2362.771	
	SCLC				3789.827
Test Statistic		2.801	4.317	b	b
Sig. (2-sided test)		0.094	0.229		
Adjusted Sig. (2-sided test)		0.293	0.366		
Homogeneous Subsets based on patient groups (SCC-Ag)					
		Subset			
		1	2	3	
Sample[a]	Benign tumors	1889.202			
	Other malignancies	2022.307			
	Healthy controls	2031.571			
	OBNTD	2169.770	2169.770		
	SCLC		2273.520		
	Pulmonary infections		2324.550		
	NSCLC			2572.006	
Test Statistic		3.655	1.942	b	
Sig. (2-sided test)		0.301	0.379		
Adjusted Sig. (2-sided test)		0.466	0.671		
Homogeneous Subsets based on patient groups (CEA)					
		Subset			
		1	2	3	
Sample[a]	Healthy controls	1820.414			
	Benign tumors	1961.500			
	Other malignancies	1962.349			
	OBNTD	1963.723			
	Pulmonary infections		2305.249		
	NSCLC			2844.898	
	SCLC			3039.289	
Test Statistic		3.386	b	1.869	
Sig. (2-sided test)		0.336		0.172	
Adjusted Sig. (2-sided test)		0.511		0.483	

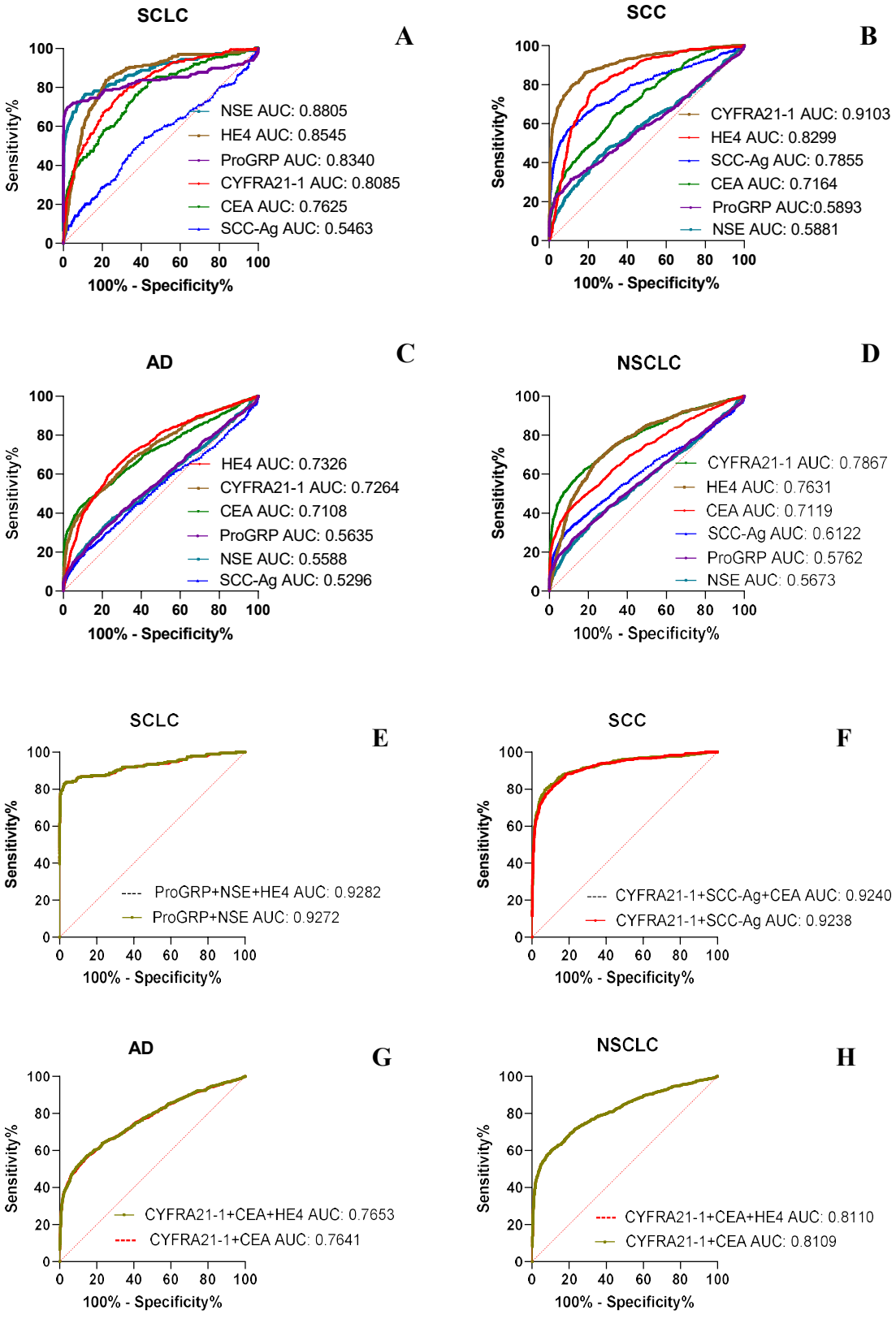
Supplementary Table S2. ***Continued ...***

Homogeneous subsets based on patient groups (CYFRA21-1)					
		Subset			
		1	2	3	4
Sample[a]	Healthy controls	1656.685			
	Benign tumors	1868.911	1868.911		
	Other malignancies		2260.500	2260.500	
	Pulmonary infections			2284.836	
	OBNTD			2358.930	
	NSCLC				3065.557
	SCLC				3075.228
Test Statistic		1.925	3.238	0.212	2.352
Sig. (2-sided test)		0.165	0.072	0.899	0.125
Adjusted Sig. (2-sided test)		0.469	0.230	0.995	0.374
Homogeneous subsets based on patient groups (HE4)					
		Subset			
		1	2	3	4
Sample[a]	Healthy controls	1550.257			
	Benign tumors		2169.958		
	Other malignancies		2353.311		
	OBNTD		2560.102		
	Pulmonary infections			2941.168	
	NSCLC			2967.109	
	SCLC				3410.228
Test Statistic		b	3.752	0.002	b
Sig. (2-sided test)			0.153	0.966	
Adjusted Sig. (2-sided test)			0.322	1.000	

Homogeneous subsets are based on asymptotic significances. The significance level is 0.05.
[a]Each cell shows the sample average rank of patient groups.
[b]Unable to compute because the subset contains only one sample.



Supplementary Figure S1. Distribution of serum levels of 6 lung cancer biomarkers.



Supplementary Figure S2. Diagnostic performance of lung cancer biomarkers: single marker vs. combinatory analysis (ROC curve).