Life Sciences 194 (2018) 1–6

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Thymidine kinase 1 combined with CEA, CYFRA21-1 and NSE improved its diagnostic value for lung cancer

Z.F. Jiang[a,*], M. Wang[b], J.L. Xu[c]

[a] Department of Respiratory Medicine, National Key Clinical Specialist Construction Programs of China, the First Affiliated Hospital of Anhui Medical University, Jixi Road 218, Hefei 230022, Anhui, PR China
[b] Department of Respiratory Medicine, Anhui Chest Hospital, Jixi Road 397, Hefei 230022, Anhui, PR China
[c] Department of Pulmonary, Shanghai Chest Hospital, Shanghai JiaoTong University, Shanghai 200030, PR China

ARTICLE INFO

Keywords:
Lung cancer
TK1
CEA
CYFRA21-1
NSE

ABSTRACT

Aims: Thymidine kinase 1 (TK1) is a tumor biomarker in human malignancies. The purpose of this study was to evaluate the diagnostic efficiency of this marker for lung cancer using the combined analysis of carcinoembryonic antigen (CEA), cytokeratin-19 fragment (CYFRA21-1), neuron specific enolase (NSE) and TK1.

Main methods: From 2013 to 2014, 147 patients with lung cancer and 228 patients with lung benign diseases who were admitted to our hospital were reviewed. Peripheral blood samples were collected for the detection of TK1, CEA, CYFRA21-1 and NSE. The diagnostic value of each marker was analyzed using receiver operating characteristic (ROC) curves and logistic regression equations.

Key findings: The serum levels of TK1, CEA, CYFRA21-1 and NSE were significantly higher than those in patients with lung benign diseases (all $P < 0.05$). The TK1 concentration was dependent on TNM stage ($P = 0.005$). The ROC curve analyses showed that the diagnostic value of TK1 combined with CEA, CYFRA21-1 and NSE in lung cancer was significantly higher than that of each biomarker alone (all $P < 0.0001$). In addition, TK1 combined with CEA, CYFRA21-1, or NSE could also improve the diagnosis of the squamous cell carcinoma, adenocarcinoma and small cell lung cancer subtypes, respectively.

Significance: The combined detection of TK1 and the other three markers significantly improved the diagnosis of lung cancer. Furthermore, the detection of TK1 combined with that of CYFRA21-1, CEA or NSE increased the diagnostic value of TK1 for lung squamous cell carcinoma, adenocarcinoma and SCLC, respectively.

1. Introduction

Lung cancer is the leading cause of cancer-related death worldwide. In China in 2010, 605,900 patients were diagnosed with and 486,600 patients died of lung cancer [1]. Despite advances in chemotherapy, radiotherapy and surgical treatment, the prognosis of this disease remains poor [2]. Therefore, early detection of lung cancer is of great importance, and the detection of a lung cancer biomarker is an effective and common approach by which individuals can be screened for lung cancer.

Carcinoembryonic antigen (CEA) was first described by Gold and Freedman in 1965 as an antigen present in gastrointestinal carcinoma cells [3]. Elevated expression of CEA was also found in patients with lung cancer, especially in those with adenocarcinoma [4]. The CYFRA21-1 protein, which is a fragment of cytokeratin subunit 19, was first reported in 1993 [5]. CYFRA21-1 over-expression has been observed in lung cancer, colorectal cancer and bladder cancer, and especially in squamous cell carcinoma [6–8]. Neuron specific enolase (NSE) is generally recognized as a marker that can be used in the diagnosis of small cell lung cancer (SCLC) [9]. Normal reference values of CEA, CYFRA21-1 and NSE are < 5.0 ng/mL, < 3.3 ng/mL and > 13.0 ng/mL, respectively.

Although serum levels of CEA, CYFRA21-1 and NSE have been extensively used as tumor markers for the diagnosis of lung adenocarcinoma, squamous cell carcinoma and SCLC, respectively, false -positive results often occur due to infections, benign tumors, and pregnancy, among other factors [10].

Thymidine kinase 1 (TK1) is a biomarker of proliferation that is associated with the salvage pathway of DNA precursor synthesis. The expression of TK1 is S-phase-dependent, and high levels of TK1 have

Abbreviations: TK1, Thymidine kinase 1; CEA, Carcinoembryonic antigen; NSE, Neuron specific enolase; SCLC, Small cell lung cancer; ROC, Receiver operating characteristic; AUC, Areas under the ROC curve; TNM, tumor-node-metastasis classification

* Corresponding author.
E-mail address: 1611601838@qq.com (Z.F. Jiang).

https://doi.org/10.1016/j.lfs.2017.12.020
Received 17 October 2017; Received in revised form 9 December 2017; Accepted 12 December 2017
Available online 14 December 2017

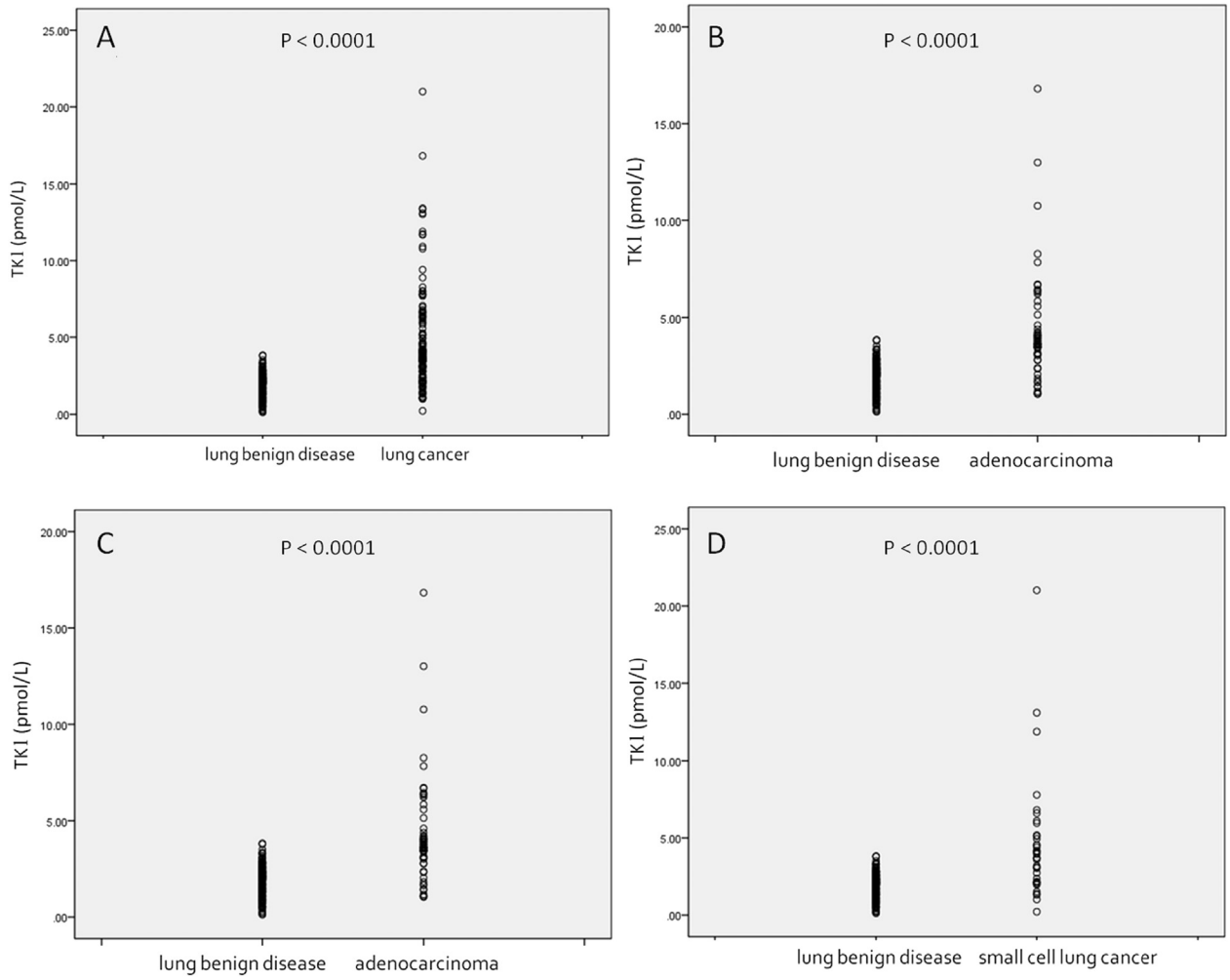

Fig. 1. The serum levels of TK1 in the peripheral blood from patients with lung cancer (A), squamous cell carcinoma (B), adenocarcinoma (C) and small cell lung cancer (D) compared with benign lung disease group.

Table 1
Relationships between the TK1 concentration and clinic-pathologic characteristics in 147 lung cancer patients. Data were presented as Median (P_{25}-P_{75}).

Items	Cases (n)	TK1 (pmol/L)	P-value
Gender			0.244
Male	111	3.87 (2.75–5.96)	
Female	36	3.66 (2.15–4.91)	
Age, year			0.805
< 60	54	3.82 (2.86–4.50)	
≥ 60	92	3.75 (2.38–6.35)	
Pathological type			0.477
Squamous	51	4.03 (2.56–6.97)	
Adenocarcinoma	59	3.63 (3.03–4.60)	
Small cell lung cancer	37	3.72 (2.15–5.15)	
TNM stage			0.005
I + II	32	2.85 (1.80–4.16)	
III + IV	115	3.94 (3.08–6.10)	

been observed in malignant cells [11]. Serological TK1 can be a useful marker to detect the early development of any type of malignancy [12]. Korkmaz et al. demonstrated a significant correlation between serum TK1 level and maximum uptake by primary tumors in positron emission tomography (PET) scans [13]. Furthermore, TK1 activity was also considered as a useful marker for assessment tumor cell proliferation in breast and colorectal cancer [14]. A previous analysis showed that a serum TK1 level of > 2.0 pmol/L might indicate a risk for the development of cancer [15].

Hence, the combined detection of various tumor markers is urgently needed to enhance the diagnosis of lung cancer. The present study was conducted to investigate the diagnostic value of the combined detection of CEA, CYFRA21-1, NSE and TK1 in the diagnosis of lung cancer.

2. Patients and methods

2.1. Patients

This retrospective study reviewed the clinical data of 147 patients with lung cancer and 228 patients with lung benign diseases at the Department of Geriatric Pulmonary in the First Affiliated Hospital of Anhui Medical University between 2013 and 2014. The study protocol was approved by the Medical Ethics and Human Clinical Trial Committee of Anhui Medical University.

The diagnosis of lung cancer was based on the pathologic data obtained by bronchofiberscope or CT-guided lung biopsy and was confirmed by two pathologists. Patients with lung cancer were evaluated according to the revised tumor-node-metastasis (TNM) classification [16]. Lung benign diseases included pneumonia, and chronic obstructive pulmonary disease, among others.

Peripheral blood samples were obtained from all the patients after diagnosis. Serum samples were separated by centrifugation at 3000 rpm for 10 min and were stored at − 20 °C until analysis. The TK1 assay was performed using a commercial kit, based on an enhanced chemiluminescence dot blot assay (SSTK Biotech Ltd., Shenzhen, China). Briefly,

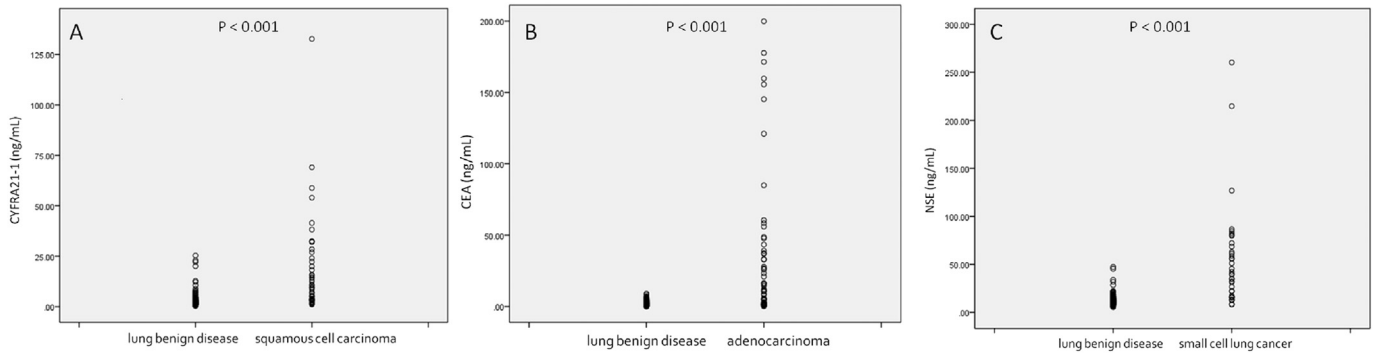

Fig. 2. The serum levels of CYFRA21-1 (A), CEA (B) and NSE (C) in the peripheral blood from patients with squamous cell carcinoma (n = 51), adenocarcinoma (n = 59) and small cell lung cancer (n = 31) compared with benign lung disease group, respectively.

Table 2
The ROC analysis of CYFRA21-1, CEA, NSE and TK1 in diagnosing lung cancer and benign lung disease.

Items	CYFRA21-1	CEA	NSE	TK1	Combination
Optimal cut-off	3.26 (ng/mL)	5.28 (ng/mL)	14.95 (ng/mL)	2.96 (pmol/L)	–
Sensitivity	0.680	0.408	0.646	0.707	0.864
Specificity	0.746	0.947	0.820	0.947	0.947
Younden's index	0.426	0.356	0.466	0.655	0.811
SE	0.0266	0.0303	0.0259	0.0229	0.0126
AUC (95% CI)	0.755 (0.708–0.798)	0.684 (0.634–0.731)	0.776 (0.731–0.817)	0.849 (0.808–0.883)	0.946 (0.918–0.966)
P-value[a]	P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001	–

[a] Comparing the area under ROC curve of combination to single tumor marker in lung cancer group vs. benign lung disease group.

3 μL serum were directly applied onto a nitrocellulose membrane. The serum samples were probed with human anti-TK1 chicken immunoglobulin Y antibody. Varying concentrations of TK1-pepetide (20, 6.6 and 2.2 pmol/L) were used as an extrapolation standard. The spot intensities on the membrane were determined by a CIS-l Imaging System (SSTK Biotech Ltd.). From the intensities of the TK1 standard of known concentrations, the TK1 concentration was calculated and expressed in pmol/L. The coefficient of variation was < 10%. Levels of CEA, NSE, and CYFRA21-1 were detected in serum specimens with a double-antibody sandwich magnetic particle chemiluminescent method using matched kits with the Roche (e601, Basel, Switzerland) automatic electrochemical luminescent immunoassay analyzer per manufacturer's instructions.

2.2. Statistical analyses

The analyses were performed using the SPSS 22.0 software package. Data are presented as the Median (P_{25}-P_{75}) for abnormal distribution data. The Kruskal-Wallis test was used to assess the correlation between TK1 concentration and pathological type, whereas the Mann-Whitney was used to assess differences in TK1 concentration according to other clinic-opathologic variables, including gender (male vs. female), age (≥ 60 vs. < 60) and TNM stage (I + II vs. III + IV). The diagnostic accuracy of CEA, CYFRA21-1, NSE and TK1 for lung cancer was evaluated using receiver operating characteristic (ROC) curves. An areas under the ROC curve (AUC) value close to 1 represents good diagnostic accuracy, whereas poor diagnostic accuracy is indicated by AUC values as low as 0.5. The comparison among AUCs was evaluated using logistic regression analysis using MedCalc 14.8.1 (MedCalc Software bvba). The ROC curve was generated using SPSS 22.0 package. A P-value of < 0.05 was considered statistically significant.

3. Results

3.1. Study population

Among the 147 patients with lung cancer who are involved in the present study, 111 were male and 36 were female, with a mean age of 61.36 ± 10.91 (range, 26–87 years). Regarding the 228 patients with lung benign diseases, 135 were male and 93 were female, with a mean age of 62.56 ± 14.90 (range, 14–98 years).

3.2. Serum levels of tumor markers in patients with lung cancer

Among the lung cancer patients, the concentration of TK1 was 3.75 (2.56–5.84) pmol/L, which was significantly higher than that in patients with benign lung disease (P < 0.01; Fig. 1-A). With respect to the different pathological types of lung cancer, a marked increase in the TK1 concentration was observed in cases of squamous cell carcinoma (P < 0.001; Fig. 1-B), adenocarcinoma (P < 0.001; Fig. 1-C) and SCLC (P < 0.001; Fig. 1-D) compared to case of benign lung disease.

Furthermore, the TK1 concentration in lung cancer patients was not associated with gender (P = 0.244), age (P = 0.805) or pathological type (P = 0.477; Table 1). Interestingly, the concentration of TK1 was dependent on the TNM stage (P = 0.005), as evidenced by higher TK1 concentrations in cancer cases at an advanced TNM stage (III + IV) compared with those at an early stage (I + II) (Table 1).

The serum level of CYFRA21-1 in cases of squamous cell carcinoma was higher than that in cases of benign lung disease (Fig. 2-A; P < 0.05). Similar results were also observed for CEA in adenocarcinoma (Fig. 2-B; P < 0.05), and for NSE in SCLC (Fig. 2-C; P < 0.05).

3.3. The diagnostic value of the TK1 in lung cancer

The ROC curves for the analyses of TK1, CYFRA21-1, CEA and NSE in patients with lung cancer were plotted. The AUCs of the four tumor markers were as follows: 0.849 (95% CI: 0.808–0.883) for TK1; 0.755 (95% CI: 0.708–0.798) for CYFRA21-1; 0.684 (95% CI: 0.634–0.731) for TK1; CEA and 0.776 (95% CI: 0.731–0.817) for NSE (Table 2). When TK1, CYFRA21-1, CEA and NSE were combined and all included in the diagnosis, the AUC was 0.946 (95% CI: 0.918–0.966, P < 0.0001; Fig. 3-A, Table 2).

The sensitivity and specificity of the four tumor markers were different when they were applied separately in the diagnosis of lung

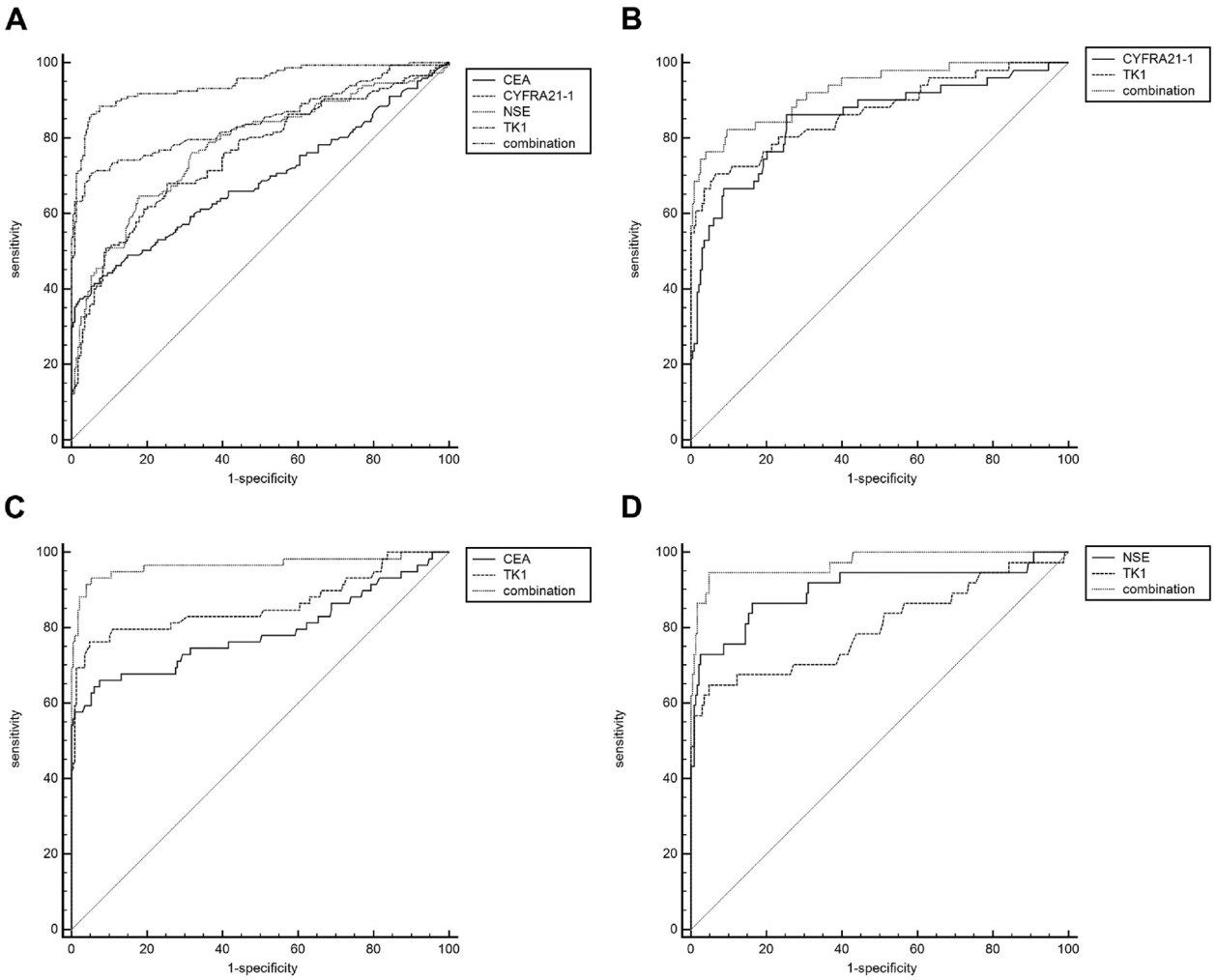

Fig. 3. ROC curves of TK1, CYFRA21-1, CEA, NSE and the combination in patients with lung cancer vs. patients with lung benign diseases (A). ROC curves of TK1, CYFRA21-1 and the combination in patients with squamous cell carcinoma vs. patients with lung benign disease (B). ROC curves of TK1, CEA and the combination in patients with lung adenocarcinoma vs. patients with lung benign disease (C). ROC curves of TK1, NSE and the combination in patients with small cell lung cancer vs. patients with lung benign disease (D).

Table 3
The ROC of the conjoint analysis of CYFRA21-1 and TK1 in lung squamous cell carcinoma.

Items	CYFRA21-1	TK1	CYFRA21-1 + TK1
Optimal cut-off	3.71 (ng/mL)	3.03 (pmol/L)	–
Sensitivity	0.612	0.701	0.864
Specificity	0.807	0.952	0.947
Younden's index	0.419	0.652	0.811
SE	0.0343	0.0328	0.0218
AUC (95% CI)	0.849 (0.802–0.889)	0.869 (0.824–0.906)	0.928 (0.891–0.955)
P-value[a]	0.0401	0.0060	–

[a] Comparing the area under ROC curve of combination to single tumor marker in lung squamous cancer vs. benign lung disease group.

Table 4
The ROC of the conjoint analysis of CEA and TK1 in lung adenocarcinoma.

Items	CEA	TK1	CEA + TK1
Optimal cut-off	4.94 (ng/mL)	3.03 (pmol/L)	–
Sensitivity	0.661	0.763	0.932
Specificity	0.925	0.952	0.947
Younden's index	0.586	0.714	0.880
SE	0.0428	0.0357	0.0178
AUC(95% CI)	0.787 (0.735–0.833)	0.861 (0.815–0.899)	0.966 (0.938–0.984)
P-value[a]	$P < 0.0001$	$P < 0.0001$	–

[a] Comparing the area under ROC curve of combination to single tumor marker in lung adenocarcinoma group vs. benign lung disease group.

cancer. TK1 showed the highest sensitivity (0.707) and specificity (0.947). When the detection of TK1 was combined with that of CYFRA21-1, CEA and NSE, the sensitivity increased to 0.864, but the specificity was unchanged (Table 2).

3.4. The diagnostic value of the combination of TK1 and the other three tumor markers for lung cancer subtypes

The 147 cases of patients with lung cancer were classified into three groups according to their pathological types: squamous cell carcinoma, adenocarcinoma and SCLC. The ROC curves of certain tumor markers combined with TK1 were plotted. Out of 51 patients with squamous cell carcinoma, the AUC of TK1 + CYFRA21-1 was 0.928 (95%CI: 0.891–0.955), which was significantly higher than that of CYFRA21-1 alone (0.849, 95%CI: 0.802–0.889, $P = 0.0401$; Fig. 3-B, Table 3). Similarly, the AUC values of TK1 + CEA and TK1 + NSE were also markedly higher than those for CEA and NSE alone in the diagnosis of adenocarcinoma ($P < 0.0001$; Fig. 3-C, Table 4) and SCLC ($P = 0.0410$, Fig. 3-D, Table 5).

Table 5
The ROC of the conjoint analysis of NSE and TK1 in small cell lung cancer.

Items	NSE	TK1	NSE + TK1
Optimal cut-off	15.51 (ng/mL)	3.04 (pmol/L)	–
Sensitivity	0.865	0.649	0.946
Specificity	0.838	0.952	0.952
Younden's index	0.703	0.600	0.898
SE	0.0363	0.0501	0.0153
AUC (95% CI)	0.901 (0.859–0.934)	0.801 (0.748–0.847)	0.972 (0.944–0.988)
P-value[a]	0.0410	0.0005	–

[a] Comparing the area under ROC curve of combination to single tumor marker in small cell lung cancer group vs. benign lung disease group.

The sensitivity and specificity of these four serum tumor markers were different when they were applied separately in the diagnosis of the lung cancer subtypes described above. CYFRA21-1 showed a sensitivity of 0.612 and a specificity of 0.807 in squamous cell carcinoma. When TK1 was used in combination with CYFRA21-1, the sensitivity in the screening for squamous cell carcinoma was markedly increased to 0.864, whereas the specificity increased to 0.947 (Table 3). Similar results were also obtained for CEA and NSE and are shown in Table 4 and Table 5.

4. Discussion

To improve the diagnosis of lung cancer, many tumor markers, including CYFRA21-1, CEA and NSE, have been intensively evaluated and have been widely used in the diagnosis of lung cancer. However, each marker has its own specificity and sensitivity, which might lead to limitations in the diagnosis. The combined detection of tumor markers maybe of great importance in the diagnosis of tumor [17–19]. TK1 has also been demonstrated to have its diagnostic value in lung cancer. In the present study, we demonstrated for the first time that the detection of TK1 combined with that of CYFRA21-1, CEA and NSE could significantly increase the AUC and the sensitivity.

TK1 has been regarded as a tumor marker for the diagnosis and prognosis of various tumors including breast, lung, prostate, and liver [20–22]. A previous study by He E et al. reported that TK1 expression was elevated in patients with lung cancer according to a chemiluminescent dot blot assay and immunohistochemistry [23]. That study also demonstrated that the level of TK1 was higher in patients with lung cancer than in patients with benign lung diseases. In the current study, our results showed a higher level of TK1 in stage III + IV lung cancers than in stage I + II lung cancers, which was in agreement with the work by Chen Y and Li HX et al. [24,25]. These results indicate that TK1 may play an important role in the occurrence and development of lung cancer.

ROC analysis has been used extensively to compare the diagnostic value of tumor markers. The AUC is considered a quantitative measure of the discrimination power of tumor markers in the differentiation of lung cancer cases from benign lung diseases [26]. The ROC curve showed a sensitivity of 0.707, a specificity of 0.947 and an optimal cut-off value of 2.96 pmol/L for serum TK1. Furthermore, the combination of TK1 with CYFRA21-1, CEA and NSE significantly improved the diagnostic sensitivity (0.864), but had no influence on the specificity. These results indicate that combined detection was helpful in the diagnosis of lung cancer.

CYFRA21-1, a fragment of cytokeratin 19, is mainly expressed in tumor cells of epithelial origin and can be used as a marker for epithelial cancers. According to one study, the level of CYFRA21-1 in squamous cell carcinoma was higher than that in adenocarcinoma and SCLC [27]. In the present study, the CYFRA21-1 level in patients with lung squamous cell carcinoma was significantly higher than those in patients with adenocarcinoma and SCLC. The sensitivity of CYFRA21-1 in the diagnosis of squamous cell carcinoma was 0.612 and the specificity was 0.807, which is in agreement with the results of Chen et al. [28]. Furthermore, in the 51 cases of squamous cell carcinoma, the ROC curve analysis demonstrated that TK1 + CYFRA21-1 had a significantly larger AUC (0.928, 95%CI: 0.891–0.955) and an increased sensitivity of 0.864 compared with CYFRA21-1 alone, which suggests a better diagnostic value of TK1 + CYFRA21-1 for lung squamous cell carcinoma. Furthermore, TK1 combined with CEA or NSE was also demonstrated to have a markedly improved diagnostic value for adenocarcinoma and SCLC, respectively.

Several limitations of this study must be addressed, such as its retrospective nature and the insufficient cases of each lung cancer subtype. Despite these limitations, this study did demonstrate that the detection of TK1 combined with CYFRA21-1, CEA and NSE significantly increased the AUC and the sensitivity in the diagnosis of lung cancer. Furthermore, the diagnostic value of TK1 was markedly increased when its detection was combined with that of CYFRA21-1, CEA or NSE for lung squamous cell carcinoma, adenocarcinoma and SCLC, respectively. It is recommended that a combination of tumor markers should be tested to obtain a more reliable diagnosis of lung cancer.

References

[1] W. Chen, R. Zheng, H. Zeng, S. Zhang, Epidemiology of lung cancer in China, Thorac. Cancer 6 (2015) 209–215.
[2] P. Mazzone, T. Mekhail, Current and emerging medical treatments for non-small cell lung cancer: a primer for pulmonologists, Respir. Med. 106 (2012) 473–492.
[3] P. Gold, S.O. Freedman, Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques, J. Exp. Med. 121 (1965) 439–462.
[4] K. Matsuoka, S. Sumitomo, N. Nakashima, D. Nakajima, N. Misaki, Prognostic value of carcinoembryonic antigen and CYFRA21-1 in patients with pathological stage I non-small cell lung cancer, Eur. J. Cardiothorac. Surg. 32 (2007) 435–439.
[5] P. Stieber, H. Bodenmuller, D. Banauch, U. Hasholzner, A. Dessauer, B. Ofenloch-Hahnle, D. Jaworek, A. Fateh-Moghadam, Cytokeratin 19 fragments: a new marker for non-small-cell lung cancer, Clin. Biochem. 26 (1993) 301–304.
[6] B. Wang, Y.J. He, Y.X. Tian, R.N. Yang, Y.R. Zhu, H. Qiu, Clinical utility of haptoglobin in combination with CEA, NSE and CYFRA21-1 for diagnosis of lung cancer, Asian Pac. J. Cancer Prev. 15 (2014) 9611–9614.
[7] D.S. Thomas, E.O. Fourkala, S. Apostolidou, R. Gunu, A. Ryan, I. Jacobs, U. Menon, W. Alderton, A. Gentry-Maharaj, J.F. Timms, Evaluation of serum CEA, CYFRA21-1 and CA125 for the early detection of colorectal cancer using longitudinal preclinical samples, Br. J. Cancer 113 (2015) 268–274.
[8] L.I. Kuang, W.J. Song, H.M. Qing, S. Yan, F.L. Song, CYFRA21-1 levels could be a biomarker for bladder cancer: a meta-analysis, Genet. Mol. Res. 14 (2015) 3921–3931.
[9] L.G. Jorgensen, K. Osterlind, J. Genolla, S.A. Gomm, J.R. Hernandez, P.W. Johnson, J. Lober, T.A. Splinter, M. Szturmowicz, Serum neuron-specific enolase (S-NSE) and the prognosis in small-cell lung cancer (SCLC): a combined multivariable analysis on data from nine centres, Br. J. Cancer 74 (1996) 463–467.
[10] R. Zimmerman, B. Wahren, F. Edsmyr, Assessment of serial CEA determinations in urine of patients with bladder carcinoma, Cancer 46 (1980) 1802–1809.
[11] K.K. Jagarlamudi, L.O. Hansson, S. Eriksson, Breast and prostate cancer patients differ significantly in their serum thymidine kinase 1 (TK1) specific activities compared with those hematological malignancies and blood donors: implications of using serum TK1 as a biomarker, BMC Cancer 15 (2015) 66.
[12] Z. Chen, H. Zhou, S. Li, E. He, J. Hu, J. Zhou, S. Skog, Serological thymidine kinase 1 (STK1) indicates an elevated risk for the development of malignant tumours, Anticancer Res. 28 (2008) 3897–3907.
[13] T. Korkmaz, S. Seber, K. Okutur, G. Basaran, F. Yumuk, F. Dane, T. Ones, O. Polat, O.C. Madenci, G. Demir, N.S. Turhal, Serum thymidine kinase 1 levels correlates with FDG uptake and prognosis in patients with nonsmall cell lung cancer, Biomarkers 18 (2013) 88–94.
[14] M. Bolayirli, C. Papila, G.G. Korkmaz, B. Papila, F. Aydogan, A. Karatas, H. Uzun, Serum thymidine kinase 1 activity in solid tumor (breast and colorectal cancer) patients treated with adjuvant chemotherapy, J. Clin. Lab. Anal. 27 (2013) 220–226.
[15] Z.H. Chen, S.Q. Huang, Y. Wang, A.Z. Yang, J. Wen, Xu XH, Y. Chen, Q.B. Chen, Y.H. Wang, E. He, J. Zhou, S. Skog, Serological thymidine kinase 1 is a biomarker for early detection of tumours—a health screening study on 35,365 people, using a sensitive chemiluminescent dot blot assay, Sensors (Basel) 11 (2011) 11064–11080.
[16] L.T. Tanoue, F.C. Detterbeck, T.N.M. New, Classification for non-small-cell lung cancer, Expert. Rev. Anticancer. Ther. 9 (2009) 413–423.
[17] H. Ma, S. Xu, J. Yan, C. Zhang, S. Qin, X. Wang, N. Li, The value of tumor markers in the diagnosis of papillary thyroid carcinoma alone and in combination, Pol. J. Pathol. 65 (2014) 202–209.
[18] M.J. Gaspar, J. De Miguel, J.D. Garcia Diaz, M. Diez, Clinical utility of a

combination of tumour markers in the diagnosis of malignant pleural effusions, Anticancer Res. 28 (2008) 2947–2952.
[19] J. Karl, N. Wild, M. Tacke, H. Andres, U. Garczarek, W. Rollinger, W. Zolg, Improved diagnosis of colorectal cancer using a combination of fecal occult blood and novel fecal protein markers, Clin. Gastroenterol. Hepatol. 6 (2008) 1122–1128.
[20] B. Nisman, T. Allweis, L. Kaduri, B. Maly, S. Gronowitz, T. Hamburger, T. Peretz, Serum thymidine kinase 1 activity in breast cancer, Cancer Biomark 7 (2010) 65–72.
[21] M. Hallek, I. Langenmayer, C. Nerl, W. Knauf, H. Dietzfelbinger, D. Adorf, M. Ostwald, R. Busch, I. Kuhn-Hallek, E. Thiel, B. Emmerich, Elevated serum thymidine kinase levels identify a subgroup at high risk of disease progression in early, nonsmoldering chronic lymphocytic leukemia, Blood 93 (1999) 1732–1737.
[22] P. Broet, S. Romain, A. Daver, G. Ricolleau, V. Quillien, A. Rallet, B. Asselain, P.M. Martin, F. Spyratos, Thymidine kinase as a proliferative marker: clinical relevance in 1,692 primary breast cancer patients, J. Clin. Oncol. 19 (2001) 2778–2787.
[23] E. He, Xu XH, H. Guan, Y. Chen, Z.H. Chen, Z.L. Pan, L.L. Tang, Hu GZ, Y. Li, M. Zhang, J. Zhou, S. Eriksson, T. Fornander, S. Skog, Thymidine kinase 1 is a potential marker for prognosis and monitoring the response to treatment of patients with breast, lung, and esophageal cancer and non-Hodgkin's lymphoma, Nucleosides Nucleotides Nucleic Acids 29 (2010) 352–358.
[24] Y. Chen, M. Ying, Y. Chen, M. Hu, Y. Lin, D. Chen, X. Li, M. Zhang, X. Yun, J. Zhou, E. He, S. Skog, Serum thymidine kinase 1 correlates to clinical stages and clinical reactions and monitors the outcome of therapy of 1,247 cancer patients in routine clinical settings, Int. J. Clin. Oncol. 15 (2010) 359–368.
[25] H.X. Li, D.S. Lei, X.Q. Wang, S. Skog, Q. He, Serum thymidine kinase 1 is a prognostic and monitoring factor in patients with non-small cell lung cancer, Oncol. Rep. 13 (2005) 145–149.
[26] H.L. Weiss, S. Niwas, W.E. Grizzle, C. Piyathilake, Receiver operating characteristic (ROC) to determine cut-off points of biomarkers in lung cancer patients, Dis. Markers 19 (2003) 273–278.
[27] J. Schneider, N. Bitterlich, H.G. Velcovsky, H. Morr, N. Katz, E. Eigenbrodt, Fuzzy logic-based tumor-marker profiles improved sensitivity in the diagnosis of lung cancer, Int. J. Clin. Oncol. 7 (2002) 145–151.
[28] F. Chen, X.Y. Wang, X.H. Han, H. Wang, J. Qi, Diagnostic value of Cyfra21-1, SCC and CEA for differentiation of early-stage NSCLC from benign lung disease, Int. J. Clin. Exp. Med. 8 (2015) 11295–11300.