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Original Article

Clinical value of serum tumor markers, beta 2 microglobulin and interieukin-6 in the diagnosis of patients with a solitary pulmonary nodule

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Abstract: Background: Detecting serum biomarkers is an important approach for early diagnosis of lung cancer. Identification of serum biomarkers for the early diagnosis of patients with malignant solitary pulmonary nodule (SPN) still requires further analysis. In this study, we detected the serum levels of tumor markers, beta 2 microglobulin (β2-MG) and interieukin-6 (IL-6) from patients and healthy individuals in order to find effective biomarkers in the early diagnosis of malignant SPN. This study enrolled 50 cases of patients with malignant SPN, 40 cases of patients with benign SPN and 40 cases of healthy individuals as normal control. The serum levels of the tumor markers, β2-MG and IL-6 in the three groups were detected and analyzed. The results show that the serum levels of CEA, CA-199, CK-19, SCC, IL-6, and β2-MG from the malignant SPN group were significantly higher than those from the benign SPN group and the normal control group. The positive rate of serum CEA, CK-19, SCC, IL-6, and β2-MG in the malignant SPN group was significantly higher than those in the benign SPN group and the normal control group. Logistic regression showed that CEA, CK-19, SCC, IL-6, β2-MG were all closely related to the diagnosis of SPN ($P<0.05$). ROC curves showed that the AUC of CEA, CK-19, SCC, IL-6, β2-MG, and their combination were 0.891, 0.854, 0.938, 0.912, 0.810, and 0.941 respectively. The combination of the five markers had the highest diagnostic value with AUC of 0.941. The best cut-off points of CEA, CK-19, SCC, IL-6, and β2-MG were 4.58, 2.95, 2.46, 6.07, and 2.95, respectively. The sensitivity of CEA, CK-19, SCC, IL-6, β2-MG, and their combination was 74%, 84%, 82%, 86%, 84%, and 82%, respectively, and the specificity was 85%, 65%, 75%, 85%, 55%, and 93%, respectively. Therefore, combined detection of serum CEA, CK-19, SCC, β2-MG, and IL-6 can provide important clinical value in the differential diagnosis of patients with SPN.

Keywords: Tumor markers, protein chip, solitary pulmonary nodule, β2-MG, IL-6

Introduction

Lung cancer is one of the most commonly diagnosed cancers with the highest mortality rate in the worldwide [1]. In recent years, with the increasing of environmental pollution, the incidence and mortality of lung cancer have gradually increased. In developing countries, the five year survival rate of lung cancer is below 10%. Due to the difficulties of early diagnosis of lung cancer, it is crucial to find effective methods to make differential diagnosis.

Recently, with the improvement of medical care, SPN has been detected with many radiological technologies [2]. SPN is defined as the lung parenchyma which is surrounded by opaque shadows with diameter less than 3 centimeters. However, the diameter of malignant SPN is often more than 3 centimeters. Malignant SPN includes small cell carcinoma, squamous carcinoma, adenocarcinoma, and large cell carcinoma. There are many reasons to form benign SPN, such as granuloma, inflammatory pseudotumor, sclerosing hemangioma, hamartoma, etc. At present, the main methods to differentially diagnose malignant SPN or benign SPN for clinicians are MSCT (multi-slice computer tomography), contrast enhanced computed tomography (CT) [3], percutaneous lung biopsy, and thoracoscopyresection, etc. In fact, it is very difficult to diagnose whether the SPN is malignant or benign unless a definite pathology diagnosis is obtained. Many patients are reluctant to accept invasive examination. As a result, clinicians hope to differentially diag-

Table 1. The basic characteristics of malignant SPN group, benign SPN group, and normal control group

	n	Malignant SPN group	Benign SPN group	Normal control group	p
Gender					
Male	94	35	30	29	0.439
Female	36	15	10	11	
Age (y)					
10~30	0	0	0	0	
30~50	14	3	17	16	
>50	116	47	20	16	
Mean age, mean ± SD		52.23±18.00	51.7±13.27	53.45±15.4	0.797
BMI (Kg/m^2)		23.16±3.25	26.16±1.20	21.39±2.37	0.612
Smoke history		38	24	13	0.024

nose using non-invasive examination. Therefore, it is urgent to improve the diagnostic accuracy of SPN with non-invasive methods. Protein chip is a novel serum detective method, and it has been widely used in the clinical auxiliary diagnosis. A large number of studies have shown that IL-6 is significantly related to invasion and metastasis of lung cancer, gastric cancer, cervical cancer, liver cancer, bladder cancer, colon cancer, ovarian cancer, etc. [4-6]. The serum levels of β2-MG also significantly increase in many cases such as cancer, infection, and transplant rejection. But it is still unknown whether or not tumor markers, IL-6, β2-MG, and their combination increase in patients with malignant SPN. If effective serum biomarkers of SPN can be found, differential diagnosis of SPN would be facilitated. Serum biomarker detection will not bring trauma and pain to the patients, and it is more conducive for clinical application and promotion [7]. In this study, the serum of patients with SPN and normal healthy individuals were separated, and the serum levels of tumor markers, IL-6 and β2-MG were detected and analyzed in order to find effective biomarkers to make differential diagnosis of SPN.

Data and methods

Subjects

A total of 90 cases of patients aged between 35 and 75 years old were enrolled into this study (Supplementary Data). These patients were admitted in the Surgical Department and Tumor Department of the Fourth Affiliated Hospital of Anhui Medical University due to SPN from January 2015 to June 2017. These patients all received surgery and obtained definite pathology diagnosis. They were divided into two groups as the malignant SPN group and the benign SPN group on the basis of lung cancer diagnosis guidelines. The exclusion criteria were specified as: patients with severe liver cirrhosis; patients with chronic renal failure; patients with congenital immunodeficiency; patients with diabetes. This study was approved by the Ethical Committee of the Fourth Affiliated Hospital of Anhui Medical University and agreed by all participants.

Table 1 contains basic information of all participants in this study.

Sample collection

Before the patients with malignant SPN accept chemotherapy, 10 ml peripheral venous blood samples from all participants were collected with disposable vacuum blood vessels and placed at room temperature for about 20 minutes. These blood samples were then centrifuged at 3500 rpm and the serum was obtained.

Protein chip

Quantitative measurements of tumor markers were conducted using a protein chip detective analyzer purchased from Shanghai Mingyuan Shukang Bio-Chip Co., Ltd, Shanghai, China. The protein chip kits and reagents were purchased from Shanghai Mingyuan Shukang Bio-Chip Co., Ltd. All above operations were strictly carried out according to the instruction manuals.

Chemiluminescence assays of IL-6 and β2-MG

The serum levels of IL-6 were detected by America DPC Immulite 1000 automatic Chemiluminescence analyzer and the relevant kits were also provided by DPC Biological Co. Ltd. The serum levels of β2-MG were detected by ZY-1200 automatic biochemical analyzer pur-

Table 2. Serum Levels of tumor markers, IL-6 and β2-MG in malignant SPN group, benign SPN group, and normal control group

Test items	Concentration units	Malignant SPN group	Benign SPN group	Normal Control group
CA199	U/mL	42.08±2.39	25.13±5.55	15.36±6.89
NSE	ng/mL	5.26±1.89	5.21±3.87	5.25±2.11
CEA	ng/mL	45.44±13.36	28.63±4.01	3.9±1.05
CA242	U/mL	14.31±2.05	14.19±6.74	13.93±5.26
CA72-4	U/mL	7.15±2.88	7.02±3.41	6.99±4.19
β-HCG	mIU/mL	2.00±1.77	1.95±1.98	1.89±2.66
AFP	ng/mL	15.06±2.42	14.88±1.76	14.93±2.30
SCC	ng/mL	2.55±0.15	2.26±0.15	1.2±2.67
C-PSA	ng/mL	3.08±1.25	2.87±0.74	2.89±1.24
CA125	U/mL	25.66±2.09	25.41±3.59	25.60±1.67
CK19	ng/mL	4.33±1.23	2.73±0.80	2.68±0.87
CA15-3	U/mL	39.41±4.32	9.67±5.19	5.23±4.72
IL-6	pg/mL	8.30±4.10	5.39±3.33	2.35±2.10
β2-MG	mg/L	3.55±2.88	1.37±0.78	1.21±0.16

Abbreviations: AFP, alpha fetoprotein; CEA, carcinoembryonic antigen; β-HCG, β-human chorionic gonadotropin; C-PSA, combined prostatespecific antigen; CA199, carbohydrate antigen 199; CA125, carbohydrate antigen 125; CK19, cytokeratin 19; NSE, neron specific enolase; SCC, squamous cell carcinoma antigen; IL-6, interieukin-6; β2-MG, beta 2 microglobulin.

Table 3. The positive rate of tumor markers, IL-6 and β2-MG in malignant SPN group, benign SPN group, and normal control group

Test items	Malignant SPN group	Benign SPN group	Normal Control group
CA199	10 (20.00)	8 (20.00)	1 (2.50)
NSE	6 (12.00)	5 (12.50)	0 (0.00)
CEA	21 (42.00)	3 (7.50)	0 (0.00)
CA242	4 (8.00)	3 (7.50)	0 (0.00)
CA72-4	5 (10.00)	4 (10.00)	0 (0.00)
β-HCG	4 (8.00)	3 (7.50)	0 (0.00)
AFP	1 (2.00)	1 (2.50)	0 (0.00)
SCC	37 (74.00)	3 (7.50)	0 (0.00)
C-PSA	5 (10.00)	4 (10.00)	0 (0.00)
CA125	1 (2.00)	1 (2.50)	0 (0.00)
CK19	38 (76.00)	8 (20.00)	0 (0.00)
CA15-3	6 (12.00)	5 (12.5)	0 (0.00)
IL-6	45 (90.00)	10 (40.00)	0 (0.00)
β2-MG	32 (64.00)	5 (12.50)	0 (0.00)

chased from Shanghai Kehua Biological Co., Ltd. All above operations were strictly carried out according to the instruction manual.

The evaluation method

The results were considered positive when the CA19-9 concentration was higher than 35 U/mL, NSE concentration was higher than 13 ng/mL, CEA concentration was higher than 5.0 ng/mL, CA242 concentration was higher than 20 U/mL, CK19 concentration was higher than 3.3 ng/mL, β-HCG concentration was higher than 3 mIU/mL, AFP concentration was higher than 20 ng/mL, SCC concentration was higher than 2.5 ng/mL, C-PSA concentration was higher than 4.0 ng/mL, CA125 concentration was higher than 35 U/mL, CA72-4 concentration was higher than 6.9 U/mL, and CA15-3 concentration was higher than 35 U/mL. The IL-6 concentration was higher than 5.9 pg/ML and the β2-MG concentration was higher than 3 mg/L.

Statistical analysis

SPSS19.0 software was used for statistical analysis. The level of the serum tumor markers, IL-6 and β2-MG, in two given groups were compared using independent-samples T-test. The statistical significance of counted data was analyzed using the Chi-square test. Logistic regression was performed to identify independent factors for prediction of malignant or benign SPNROC curve and was used to analyze the diagnostic value of serum tumor markers, IL-6 and β2-MG levels were used for differentiating malignant and benign SPN. The best cut-off points, sensibility, specificity, PPV, and NPV of serum tumor markers, IL-6 and β2-MG, were calculated respectively. Differences were statistically significant when the *P* value was less than 0.05.

Results

Characteristics of participants

In total, 130 subjects consisting of 94 men and 36 women were enrolled in this study. Study groups include malignant SPN (n=50), benign SPN (n=40), and normal individuals (n=40). The malignant SPN group consisted of 35 men and 15 women (age between 10 and 30 years: n=0, age between 30 and 50years: n=3, age >50 years: n=47) with an average age of 52.23±18.00 years old. The benign SPN group consisted of 30 men and 10 women (age

Table 4. Logistic regression analysis of serum levels of CEA, CK-19, SCC, IL-6, β2-MG in the malignant SPN group and the benign SPN group

Test items	β	SE	P	Asymptotic 95% confidence interval
CEA	0.266	0.057	<0.001	0.380~0.152
CK-19	0.093	0.025	<0.001	0.043~0.142
SCC	0.470	0.807	0.048	1.135~2.075
IL-6	0.392	0.126	0.003	0.141~0.643
β2-MG	0.034	0.053	0.008	0.071~0.138
Constant	-1.525	0.624	<0.001	6.279~8.772

between 10 and 30 years: n=0, age between 30 and 50 years: n=6, age >50 years: n=34) with an average age of 51.7±13.27 years old. The normal control group consisted of 29 men and 11 women (age between 10 and 30 years: n=0, age between 30 and 50 years: n=5, age >50 years: n=35) with an average age of 48.45±15.40 years old. The age distribution showed no significant difference between the malignant SPN group and the benign group (P>0.05). The age of the malignant SPN group was significantly higher than that of the control group (P<0.05). BMI results showed no significant difference between the malignant SPN group and the benign group (P>0.05). The number of patients with smoking history in the malignant SPN group was significantly higher than that of the *benign SPN group and the control group* (P<0.05). The demographic characteristics of the three groups are shown in **Table 1**.

Serum levels of tumor markers, IL-6 and β2-MG, in the malignant SPN group, benign SPN group, and normal control group

The average serum levels of CEA, CA-199, CK-19, SCC, IL-6, and β2-MG in the malignant SPN group were significantly higher than those in the benign SPN group and the normal control group (P<0.05) which are shown in **Table 2**. No significant difference was found in the serum levels of AFP, β-HCG, C-PSA, NSE, CA242, CA72-4, CA15-3, and CA125 between the malignant SPN group and the other groups (P>0.05).

The positive rate of tumor markers, IL-6 and β2-MG, in the malignant SPN group, the benign SPN group, and the normal group

The positive rate of serum CEA, CK-19, SCC, IL-6, and β2-MG in the malignant SPN group was significantly higher than in the benign SPN group and the normal control group (P<0.05) which are shown in **Table 3**. No significant differences were found in the positive rate of serum AFP, β-HCG, C-PSA, NSE, CA242, CA72-4, CA15-3, CA125, and CA 199 between the malignant SPN groups and the benign SPN groups (P>0.05). In **Table 2**, the average serum levels of CEA, CA-199, CK-19, SCC, IL-6, and β2-MG in the malignant SPN group were significantly higher than those in the benign SPN group and the normal control group (P<0.05). However, no significant differences were found in the positive rate of serum CA-199 between the malignant SPN groups and the benign SPN groups (P>0.05). So the positive rate can better reflect the sensitivity of those indexes *for differential diagnosis of SPN.* Logistic regression was performed to analyze the serum Levels of CEA, CK-19, SCC, IL-6, and β2-MG in the malignant SPN group and the benign SPN group.

Assuming that X1=CEA, X2=CK-19, X3=SCC, X4=IL-6, X5=β2-MG, the predictive probability value regression model for malignant SPN was constructed. Y=1/(1+EXP(0.266X1+0.093X2+0.470X3+0.392X4+0.034X5-1.525)). Logistic regression showed that CEA, CK-19, SCC, IL-6, and β2-MG are all closely related to the diagnosis of SPN (P<0.05), which are shown in **Table 4**.

ROC curves for serum levels of CEA, CK-19, SCC, IL-6, β2-MG and their combination for differential diagnosis of SPN

Due to the higher positive rate of serum CEA, CK-19, SCC, IL-6, and β2-MG in the malignant SPN group, five biomarkers and their combination were chosen to make ROC curves. ROC curves were used to analyze the diagnostic value of different indexes between the malignant SPN group and the benign SPN group (**Figure 1**, **Tables 5** and **6**).

The AUC of CEA, CK-19, SCC, IL-6, β2-MG and their combination were 0.891 (95% *CI*: 0.824~0.957), 0.854 (95% *CI*: 0.779~0.929), 0.938 (95% *CI*: 0.890~0.987), 0.912 (95% *CI*: 0.848~0.975), 0.810 (95% *CI*: 0.722~0.898) and 0.941 (95% *CI*: 0.893~0.988). The area under the curve of the combination of the five markers was the largest, so the combination of the five markers had the greatest diagnostic value. SCC had the second greatest diagnostic value with AUC of 0.938. IL-6 had the third greatest diagnostic value with AUC of 0.912. The best cut-offs of CEA, CK-19, SCC, IL-6, and

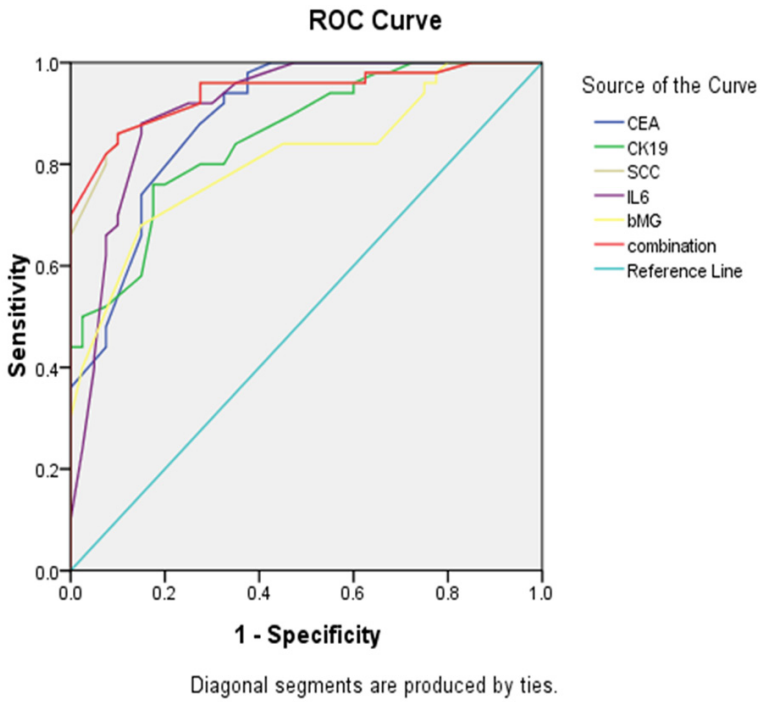

Figure 1. The ROC curves of Serum Levels of CEA, CK-19, SCC, IL-6, bβ2-MG and their combination for differential diagnosis of SPN.

Table 5. Analysis of ROC of serum levels of CEA, CK-19, SCC, IL-6, β2-MG to distinguish malignant SPN and benign SPN

Items	AUC	Standard error	P	Asymptotic 95% confidence interval
CEA	0.891	0.034	<0.001	0.824~0.957
CK-19	0.854	0.038	<0.001	0.779~0.929
SCC	0.938	0.025	<0.001	0.890~0.987
IL-6	0.912	0.032	<0.001	0.848~0.975
β2-MG	0.810	0.045	<0.001	0.722~0.898

β2-MG were 4.58, 2.95, 2.46, 6.07, and 2.95 respectively. The sensitivity of CEA, CK-19, SCC, IL-6, β2-MG and their combination associated with the corresponding cut-off points were 74%, 84%, 82%, 86%, 84%, and 82% respectively, and the specificity were 85%, 65%, 75%, 85%, 55%, and 93%, respectively. The combination of five markers had the greatest sensitivity and specificity. PPV, NPV, AUC and CI were also calculated and presented which were displayed in **Table 7**.

Discussion

Recent improvements in living standards have led more people to pay attention to their health. Due to air pollution, smoking history, family history of cancer, and other factors, a large number of people have been found to have SPN though physical examination [8]. They need further examination to distinguish whether the SPN is malignant or benign SPN. However, many people are reluctant to accept invasive screening methods such as percutaneous lung biopsy, thoracoscopic resection, etc. due to unnecessary fear and anxiety. In fact, most of the SPN are benign and only a small part of SPN is malignant. Malignant SPN could be the early stage of lung cancer or precancerous lesions, and once the malignant SPN was not detected, it will transform into lung cancer. If the malignant SPN is detected and cut instantly, the five-year survival rate will increase significantly. Regarding the clinical importance of early diagnosis of lung cancer, it is urgent for us to find an effective and non-invasive method to distinguish malignant SPN and benign SPN. Serum tumor markers are kind of biochemical substances, including AFP, CEA, CA199, CA125, CK19, NSE, and SCC, etc. Furthermore, a number of novel biomarkers such as microRNA-21, miR-1254, and miR-574-5p [9, 10] are all considered as important predictors which can reflect the existence and growth of tumors. Protein chip [11, 12] detection methods are simple and rapid and can detect 12 tumor markers at one time, including alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen 199 (CA199), carbohydrate antigen 125 (CA125), cytokeratin 19 (CK19), neuron specific enolase (NSE), squamous cell carcinoma antigen (SCC), etc. CEA is non-organ specific tumor associated antigen which is mainly used in differential diagnosis of digestive system tumor such as colorectal cancer, pancreatic cancer, cholangiocarcinoma and gastric cancer. Various studies have also showed that CEA increased significantly in lung cancers. Kim et al. found that the average serum levels of CEA in the malignant SPN group were significantly higher than in the benign SPN group (P<0.05). Similarly, in our study, we found

Table 6. Analysis of ROC of serum levels of combination of CEA, CK-19, SCC, IL-6, β2-MG to distinguish malignant SPN and benign SPN

Items	AUC	Standard error	P	Asymptotic 95% confidence interval
Combination	0.941	0.024	<0.001	0.893~0.988

Table 7. Indices of validity for serum levels of CEA, CK-19, SCC, IL-6, β2-MG and combination to distinguish malignant SPN group and benign SPN group

Items	Sensitivity	Specificity	PPV	NPV
CEA	42%	92.5%	87.5	56.06
CK-19	76%	80%	82.61	72.73
SCC	74%	92.5%	92.5	74.00
IL-6	90%	75%	81.82	88.89
β2-MG	64%	87.5%	86.49	66.04
Combination	82%	93%	93.19	80.43

good diagnostic accuracy of CEA in differential diagnosis of SPN with a specificity of 92.5%. According to related research, serum levels of CK-19 increased significantly in non-small cell lung cancer. Our study found the average serum level and positive rate of CK-19 in the malignant SPN group were significantly higher than the benign SPN group. SCC is an important tumor marker to make differential diagnosis of lung squamous carcinoma [13]. We found SCC had the second highest diagnostic value with AUC of 0.938 and the sensitivity and specificity were 74% and 92.5% respectively. In many cases such as tumors, inflammation, and the damage of organism, serum levels of IL-6 and β2-MG were found increased significantly and are thus considered as important early predictors of many cancers. In our study, we found the average serum level and positive rate of IL-6 and β2-MG in the malignant SPN group were significantly higher than those in the benign SPN group (P<0.05). The sensitivity and specificity of IL-6 were 90% and 75%, and the sensitivity and specificity of β2-MG were 64% and 87.5%. IL-6 could make a differential diagnosis of SPN with the third highest accuracy (AUC=0.912). In addition to that, the NPV of IL-6 was up to 88.89, so we can use it to avoid unnecessary invasive examination. Despite of the good performance of and diagnostic accuracy of these single markers, we also wanted to know whether the diagnostic accuracy would increase if we combined all these single markers. According to our findings the combination of CEA, CK-19, SCC, IL-6, and β2-MG could distinguish malignant SPN from benign SPN with the highest accuracy (AUC=0.941), and the high specificity of 93% to make differential diagnosis of SPN.

Conclusion

In conclusion, IL-6 and β2-MG are important early predictors of many cancers. In our study, we found the average serum level and positive rate of IL-6 and β2-MG in the malignant SPN group were significantly higher than those in the benign SPN group (P<0.05). We also found that the combination CEA, CK-19, SCC, IL-6, and β2-MG could increase the diagnosis accuracy, specificity, and sensitivity to differentiate malignant and benign SPN.

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Disclosure of conflict of interest

None.

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