RESEARCH ARTICLE

Clinical Utility of Haptoglobin in Combination with CEA, NSE and CYFRA21-1 for Diagnosis of Lung Cancer

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Abstract

Purpose: To investigate the clinical value in lung cancer of a combination of four serum tumor markers, haptoglobin (Hp), carcinoembryonic antigen (CEA), neuron specific enolase (NSE) as well as the cytokeratin 19 fragment (CYFRA21-1). Materials and Methods: Serum Hp (with immune-turbidimetric method), CEA, NSE, CYFRA21-1 (with chemiluminescence method) level were assessed in 193 patients with lung cancer, 87 patients with benign lung disease and 150 healthy controls. Differences of expression were compared among groups, and joint effects of these tumor markers for the diagnosis of lung cancer were analyzed. Results: Serum tumor marker levels in patients with lung cancer were obviously higher than those with benign lung disease and normal controls ($p<0.01$). The sensitivities of Hp, CEA, NSE and CYFRA21-1 were 43.5%, 40.9%, 23.3% and 41.5%, with specificities of 90.7%, 99.2%, 97.9% and 97.9%. Four tumor markers combined together could produce a positive detection rate of 85.0%, significantly higher than that of any single test. With squamous carcinomas, the positive detection rates with Hp and CYFRA21-1 were higher than that of other markers. In the adenocarcinoma case , the positive detection rate of CEA was higher than that of other markers. For small cell carcinomas, the positive detection rate of NSE was highest. The area under receiver operating characteristic curve (AUC^{ROC}) of Hp in squamous carcinoma (0.805) was higher than in adenocarcinoma (0.664) and small cell carcinoma (0.665). Conclusions: Hp can be used as a new serum tumor marker for lung cancer. Combination detection of Hp, CEA, NSE and CYFRA21-1 could significantly improve the sensitivity and specificity in diagnosis of lung cancer, and could be useful for pathological typing.

Keywords: Lung cancer - diagnosis - Hp - CEA - NSE - CYFRA21-1

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Introduction

Lung cancer is one of the most common malignant tumors in the world, and its morbidity and mortality are rising year by year. Early stage diagnosis of lung cancer is of great significance in clinical work. Tumor markers associated with lung cancer have been found constantly, but there is no one kind of tumor markers with high sensitivity and specificity for lung cancer. The method of joint detection of tumor markers for lung cancer early diagnosis and pathological typing was mostly used clinically. Haptoglobin (Hp) is widely existing among serums and other body fluids in human and many other mammals, its main function is to combine the free hemoglobin so as to prevent the kidneys and vasculature from oxidative damage induced by free hemoglobin, and promote the cycle of heme iron (Langlois et al., 1996; Melamed-Frank et al., 2001). Studies have pointed out that Hp has higher expressions in oral squamous carcinoma, pancreatic cancer, lung cancer and other cancer patients serum or body fluids (Chih-Ho et al., 2010; Kang et al., 2011; Farooqui et al., 2012; Jessie et al., 2013), indicating that Hp has potential clinical application value in tumor diagnosis. This research, through the haptoglobin (Hp) in combination with carcinoembryonic antigen (CEA), neuron specific enolization enzyme (NSE) and cytokeratin 21-1 (CYFRA21-1) detect, to explore the clinical value of single or multiple markers detection to the diagnosis of lung cancer, and to further evaluate the application value of Hp as biomarker to diagnose lung cancer.

Materials and Methods

Human subjects

All patients enrolled in this study with lung cancer were treated at PLA 81 hospital from November 2011 to March 2014. All lung cancer patients were by pathologically or cytologically confirmed including 81cases of squamous carcinoma (SC), 76 cases of adenocarcinoma (AC), and 36 cases of small cell carcinoma (SCC). Patients with lung benign disease were diagnosed with pulmonary tuberculosis, pulmonary infection and chronic obstructive pulmonary disease, totally 87 patients. Control group was consisted by 150 subjects who underwent healthy physical examination. Serum samples were collected from all subjects and stored at -20°C until analysis.

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Detection methods and Evaluation

Hp was detected by Immune-turbidimetric method. CEA, NSE and CYFRA21-1 were detected by Chemiluminescence immunoassay. Critical value of Hp, CEA, NSE and CYFRA21-1 was 2.0g/L, 9.8μg/L, 12μg/L and 3.5μg/L. Positive test was set if serum specimen detected greater than the above value. In combined detection, the general was judged as positive as long as any one marker was positive and the general was judged as negative when all above four markers were negative.

Statistical analysis

SPSS16.0 statistical software was used for statistical analysis. Comparison of tumor marker level in groups was conducted using rank sum test. Comparison of tumor marker positive rate in pathological types was conducted using χ^2 test. Receiver operator characteristic (ROC) curves were generated and the area under the curve (AUC) was calculated to compare the diagnostic accuracy of tested markers for predicting pathological type of lung cancer.

Results

Serum level of tumor markers in each group

Serum levels of Hp, CEA, NSE and CYFRA21-1 are summarized in Table 1. Serum level of Hp, CEA, NSE and CYFRA21-1 in patients with lung cancer was significantly higher than those with lung benign disease or control ($p<0.01$). This difference was not detected between subjects with lung benign disease and healthy controls ($p>0.05$).

Sensitivity and specificity of tumor markers and their combination for lung cancer

The sensitivity and specificity of these four serum tumor markers were different when separately conducted in the diagnosis for lung cancer. Serum Hp showed a high specificity (90.7%) but low sensitivity (43.5%). When all of HP, CEA, NSE and CYFRA21-1 were used in combination, the sensitivity in screening for lung cancer was remarkably increased to 85.0% without compromising the specificity (85.7%) (Table 2).

Table 1. Serum Levels of Hp, CEA, NSE and CYFRA21-1 in Different Groups

	n	Median(range)	Z_1*	P_1*	Z_2**	P_2**
Hp(g/L)						
Lung cancer	193	1.93(0.36~5.74)				
Lung benign disease	87	1.49(0.37~3.74)	-5.49	<0.01		
Normal control	150	0.85(0.13~2.14)			-13.09	<0.01
CEA(μg/L)						
Lung cancer	193	7.5(0.7~873.3)				
Lung benign disease	87	2.6(0.4~10.8)	-7.87	<0.01		
Normal control	150	2.3(0.1~6.7)			-10.34	<0.01
NSE(μg/L)						
Lung cancer	193	7.4(1.7~201.8)				
Lung benign disease	87	5.9(1.8~24.9)	-5.54	<0.01		
Normal control	150	3.8(0.3~9.7)			-11.12	<0.01
CYFRA21-1(μg/L)						
Lung cancer	193	2.9(0.6~251.2)				
Lung benign disease	87	2.3(0.6~13.8)	-7.09	<0.01		
Normal control	150	1.5 (0.1~4.3)			-11.28	<0.01

*Lung benign disease compared with lung cancer; **Normal control compared with lung cancer

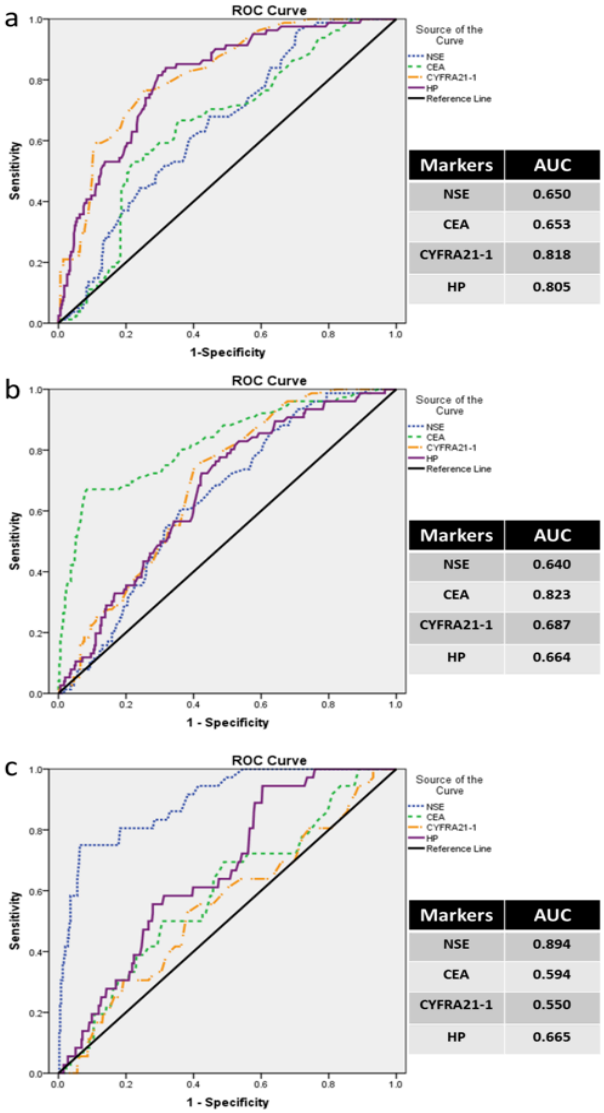

Figure 1. ROC Curves of Hp, CEA, NSE and CYFRA21-1 for Lung Cancer. a) squamous carcinoma (SC); **b)** adenocarcinoma (AC); **c)** small cell carcinoma (SCC)

Table 2. Sensitivity and Specificity (%) of Tumor Markers for Lung Cancer

Markers	Sensitivity	Specifity
Hp	43.5(84/193)	90.7(215/237)
CEA	40.9(79/193)	99.2(235/237)
NSE	26.9(52/193)	97.9(232/237)
CYFRA21-1	41.5(80/193)	97.9(232/237)
Hp+CYFRA21-1	65.8(127/193)	88.6(210/237)
Hp+CYFRA21-1+CEA	80.3(155/193)	87.8(208/237)
Hp+CYFRA21-1+CEA+NSE	85.0(164/193)	85.7(203/237)

Table 3. Comparison of Positive rate (%) of Tumor Markers for Different Pathological Type of Lung Cancer

Markers	SC	AC	SCC
Hp	54.3(44/81)	35.5(27/76)	36.1(13/36)
CEA	21.0(17/81)	67.1(51/76)	30.6(11/36)
NSE	17.3(14/81)	14.5(11/76)	75.0(27/36)
CYFRA21-1	59.3(48/81)	27.6(21/76)	30.6(11/36)
Hp+ CYFRA21-1	82.7(67/81)	52.6(40/76)	55.6(20/36)
Hp + CYFRA21-1+CEA	84.0(68/81)	80.3(61/76)	72.2(26/36)
Hp+ CYFRA21-1+CEA +NSE	85.2(69/81)	82.9(63/76)	88.9(32/36)

Diagnostic accuracy of serum Hp, CEA, NSE and CYFRA21-1 for different pathological type of lung cancer

The positive rate of four tumor markers was different when separately conducted in the diagnosis for different pathological type of lung cancer, rank from high to low, CYFRA21-1>Hp>CEA>NSE (in SC); CEA>Hp>CYFRA21-1>NSE (in AC); NSE>Hp>CEA=CYFRA21-1 (in SCC). In terms of joint detection, the positive rate of Hp+CYFRA21-1 in SC was higher than in AC and SCC (p<0.05). Hp+CYFRA21-1+CEA+NSE suggested to be a combination with highest positive rate (85.2% in SC, 82.9 in AC and 88.9% in SCC) (Table 3).

The area under the ROC curve (AUROC) was calculated to compare the accuracies achieved when using Hp, CEA, NSE and CYFRA21-1 for diagnosis of different pathological type of lung cancer (Figure 1). In SC, the AUC of Hp (0.805) and CYFRA21-1 (0.818) were higher than those of CEA (0.653) and NSE (0.650). In AC, the AUC of CEA (0.823) was higher those of Hp (0.664), NSE (0.640) and CYFRA21-1 (0.687). In SCC, the AUC of NSE (0.894) was higher than those of Hp (0.665), CEA (0.594) and CYFRA21-1 (0.550).

Discussion

The tumor makes constant substance exchange with the surrounding environment in the process of growth and proliferation. These matters secreted by tumor are not only necessary for tumor cell growth, invasion and metastasis, proteins secreted by tumor can reach the blood and other body fluids as tumor markers. Detection of tumor markers in the blood is kind of method which is quick and easy. It is particularly important in the early screening of tumor. In this study, serum levels of Hp, CEA, CYFRA21-1 and NSE in patients with lung cancer were significantly higher than those with lung benign disease group and healthy group, indicating that the four kinds of tumor markers had a certain value in diagnosis of lung cancer.

CEA is acid glycoprotein of human embryonic antigen specificity determinant, belongs to non-organ specific tumor associated antigen, the content is extremely small in the circulating blood of normal adult, the elevated degree has a closely relation with the proliferation of cancer cell, has been widely used in various types of tumors detection (Lee et al., 2013; Ding et al., 2014). The results of this study showed that the level of CEA in adenocarcinoma was significantly higher than that in squamous carcinoma and small cell cancer, indicating that CEA is the most valuable indicator for the diagnosis of lung adenocarcinoma. CYFRA21-1 is a fragment of cytokeratin 19, mainly distributed in tumor cells of epithelial origin, can be used as a marker for epithelial tumors. Serum of CYFRA21-1 was released and raised when cell dissolved. Expression of CYFRA21-1 in non-small cell lung carcinoma was higher than that in small cell lung carcinoma, in squamous carcinoma was higher than that in adenocarcinoma (Schneider et al., 2005). According to the positive rate and the AUROC showed in this study, level of CYFRA21-1 in serum of patients with Lung squamous cell carcinomas were higher than those with lung adenocarcinoma and small cell carcinoma. NSE is also called phosphoenolpyruvic invertase, and it is isoenzyme of neuron-specific enolase. NSE is over-expressed in small cell lung cancer which origins from neuroendocrineneoplasm. So NSE can be used as specific tumor marker (Juan et al., 2004) in small cell lung cancer and is important in the diagnosis of small cell lung cancer. In this study, the specificity of NSE in detection of lung cancer was higher (97.9%), but the sensitivity was lower (26.9 %). The positive rate in small cell carcinoma was significantly higher than that in squamous cell carcinoma and adenocarcinoma.

Haptoglobin (Haptoglobin, Hp) is one kind of acidoglycoprotein in serum α2 Globulin components, including three basic genetic types, namely, Hp1-1, Hp2-1 and Hp2-2, and different genotypes have different biological functions. There are some differences in combining with free hemoglobin, antibacterial effect, promoting angiogenesis and immune regulation, etc. Hp has played a role in the deterioration of epithelial cell, immunosuppression and angiogenesis in the process of cancer development, a number of studies have shown that Hp content significantly increased in patients with tumor (Fujita et al., 2014; Zhu et al., 2014). Moreover, Hp, as one kind of acute phase protein, plays an important role in the process of host anti-infection, the repairing of tissue and stabilizing internal environment, the content of serum Hp has a significant rise in pathological conditions such as infection, trauma, inflammation, tumor and myocardial infarction (MI). In addition to the above functions, Hp has also involved in oxidative stress, immune response, and so on (Asleh et al., 2014; Bertaggia et al., 2014; Tan et al., 2014). This study found that Hp level significantly increased in the lung cancer patients group. Hp positive rate was 43.5%, specificity was 90.7%. The possible reason of Hp specificity was lower than those of the other three indexes is that the Hp is still an acute phase reaction protein and will also rise in benign lung disease for stress reaction, but the risen level in benign lung disease is lower than in patients with lung cancer. Hp positive rate of squamous carcinoma patients is higher than that of adenocarcinoma patients and small cell carcinoma patients. The area under the ROC curve is 0.805 in the diagnosis of squamous cell carcinomas, indicating that Hp has high diagnostic value for lung cancer, especially for squamous carcinoma.

The study shows that the specificity is relatively high and the sensitivity is low while applying single tumor marker in the diagnosis of lung cancer. Susceptibility of four joint detection is significantly increased (85.0%) compared with single detection (p<0.05). While specificity of four joint detection is lower (85.7%) than that of single detection, but the difference has no statistical significance (p>0.05). The results indicate that joint detection is helpful to the diagnosis of lung cancer.

This study compared the positive rate of different tumor maker single and joint detection in different pathological types of lung cancer, discovered that in the diagnosis of lung squamous carcinoma, the positive rate of Hp and CYFRA21-1 joint detection has greatly increased compared with Hp or CYFRA21-1 respective single

detection. While the difference of positive rate between Hp and CYFRA21-1 joint detection (82.7%) and the positive rate of three (84.0%) or four (85.2%) joint detection is not significant. It suggests that the combination of Hp+ CYFRA21-1 was the index in identifying the squamous cell carcinomas and has important clinical significance in auxiliary diagnosis of lung squamous carcinoma.

To sum up, serum Hp, CEA, NSE and CYFRA21-1 have certain auxiliary diagnostic value to the diagnosis and pathological classification of lung cancer. Hp and CYFRA21-1 has high diagnostic value for squamous carcinoma, CEA has high diagnostic value for adenocarcinoma, and NSE has high diagnostic value for small cell carcinoma. Hp and CYFRA21-1 joint detection has larger significance for the diagnosis of lung squamous carcinoma, further confirmed that the Hp can be used as a new serum tumor marker and has a high clinical application value for lung cancer, especially in the diagnosis of lung squamous carcinoma.

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