RESEARCH ARTICLE

Application of Joint Detection of AFP, CA19-9, CA125 and CEA in Identification and Diagnosis of Cholangiocarcinoma

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Abstract

Objective: To explore the application of joint detection of serum AFP, CA19-9, CA125 and CEA in identification and diagnosis of cholangiocarcinoma (CC). Materials and Methods: The levels of serum AFP, CA19-9, CA125 and CEA of both 30 patients with CC and 30 patients with hepatocellular carcinoma (HCC) were assessed. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic effects of single and joint detection of those 4 kinds of tumor markers for CC. Results: The levels of serum CA19-9, CA125 and CEA in CC patients were higher than that in HCC patients,whereas that of serum AFP was significantly lower s. The area under ROC curve of single detection of serum AFP, CA19-9, CA125 and CEA were 0.05, 0.86, 0.84 and 0.83, with the optimal cutoff values of 15.4 ng/ml, 125.1 U/ml, 95.7 U/ml and 25.9 ng/ml, correspondingly, and the percentage correct single diagnosis was <79%. With joint detection, the diagnostic effect of combined AFP, CA19-9, CA125 and CEA was the highest, with an area under the ROC curve of 0.94 (95%CI 0.88~0.99). Conclusions: Single detection of serum CA19-9, CA125 and EA is not meaningful. The sensitivity, specificity, the rate of correct diagnosis and the area under ROC curve of joint detection of AFP, CA19-9, CA125 and CEA are highest, indicating that the joint detection of these 4 tumor markers is of great importance in the diagnosis of CC.

Keywords: Cholangiocarcinoma - hepatocellular carcinoma - tumor markers - identification and diagnosis - ROC curve

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Introduction

Primary liver cancer (PLC) primarily occurs in parenchymal hepatic cell or bile duct cell carcinomas and includes hepatocellular carcinoma (HCC), cholangiocarcinoma (CC) and cHCC-CC (Dai et al., 2013; Liu et al., 2013; Xu et al., 2013; Al-Bahrani et al., 2015). Of all, CC is a kind of PLC of primary intrahepatic biliary epithelial cells and its overall incidence accounts for 10% of PLC, with the increasing morbidity and mortality year by year (Mohammad-Alizadeh et al., 2012). In America, the morbidity of CC in the period of 1995~1999 increased by 165% compared with the period of 1975~1979, especially the morbidity of extrahepatic cholangiocarcinoma increased year after year in the period of 1992~2007 (Tyson et al., 2014). CC has a poor prognosis, and radical resection is clinically only one treatment means for it at present (Hemming et al., 2005). However, the onset of CC is hidden, with no obvious symptoms, difficult to identify it with HCC patholomorphologically. Therefore, it is hard to diagnose early CC and the rate of missed diagnosis and misdiagnosis rate are high. So far, we have not yet found specific tumor markers for CC, which brings a lot of difficulties to early diagnosis and treatment of it (Qu et al., 2012). Serum AFP, CA19-9, CA125 and CEA are common digestive tract tumor markers. Hence, this study was intended to increase the accuracy rate of diagnosis for CC by exploring clinical effects of the serum levels of those 4 tumor markers in diagnosis of CC. The results are as follows.

Materials and Methods

General data

Thirty patients with CC and 30 patients with hepatocellular carcinoma (HCC) in our hospital from Jan., 2013 to Dec., 2014 were enrolled and confirmed by laboratory examination, imaging examination and pathology. Inclusion criteria: (1) patients without special medical history in recent 1 month, such as liver damage drugs which affecting the experimental results; (2) patients without liver, kidney, lung and heart diseases and the history of the relevant surgery; (3) patients receiving liver function, AFP, CA125, CA19-9, CEA and imaging examinations including ultrasound B, CT, MRI. Of CC patients, there were 18 males and 12 females, aged 41~75

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Table 1. Comparison of the Levels of Serum AFP, CA19.9, CA125 and CEA Between CC and HCC Patients

	CC			HCC			*P*
	Median	Percentiles 25	Percentiles 75	Median	Percentiles 25	Percentiles 75	CC vs. HCC
AFP (ng/mL)	44.29	2.73	200.17	544.19	229.25	961.32	3.49E-06
CA19-9 (U/mL)	136.77	85.725	185.07	37.95	16.8725	75.44	2.66E-05
CA125 (U/mL)	109.6	49.405	201.7625	17.085	8.47	44.0225	1.93E-05
CEA (ng/mL)	11.2075	17.495	21.8575	3.48	1.185	6.9325	4.47E-04

Table 2. Area Under ROC Curve of AFP, CA19-9, CA125 and CEA

	AUC-ROC	Std. Error	*P*	95% Confidence Interval
AFP	0.05	0.02	1.38E-05	0.07~0.276
CA19-9	0.86	0.05	1.67E-06	0.77~0.95
CA125	0.84	0.05	5.86E-06	0.73~0.93
CEA	0.83	0.05	1.05E-05	0.74~0.94

years, with the average age of (57.61±6.53) years. Of HCC patients, there were 19 males and 11 patients, aged 39~82 years, with the average of (60.34±9.85) years. There was no statistical difference between general data of 2 groups such as age, gender etc., comparable (P>0.05).

Methods

The fasting venous blood (5 mL) of patients in the morning was drawn and centrifuged at 3 000 r/min for 10 min within 3 h of blood specimen collection. The super serum was drawn and subpackaged into the sterile tube at –80°C fridge for preservation. Chemiluminescence was used for detecting the levels of serum AFP, CA19-9, CA125 and CEA in Automatic biochemical analyzer.

Statistical data analysis

SPSS17.0 software package was applied for data analysis. The non-normal measurement data were presented as the median and interquartile-range (IQR). Comparison among groups was analyzed using Mann-Whitney test. A value of p<0.01 was considered to be statistical significance. The single index and combined indexes of AFP, CA19.9, CA125 and CEA was conducted to do ROC curve to evaluate the diagnostic effect of the corresponding indexes. The detecting values of AFP, CA19-9, CA125 and CEA were put into Binary Logistic regression analysis for establishing best binary classification regression model. The rate of correct diagnosis was observed by retrospective survey.

Results

Comparison of the levels of serum AFP, CA19-9, CA125 and CEA between CC and HCC patients

Four variable indexes was not accord with normal distribution and the tendency of dispersion was presented as the median and IQR. The levels of serum CA125 and CEA of CC patients were higher than those of HCC patients, but the level of serum AFP of CC patients was lower than that of HCC patients, the difference was significant (P<0.01), as shown in Table 1.

Evaluation of diagnostic effect of single detection of serum AFP, CA19-9, CA125 and CEA

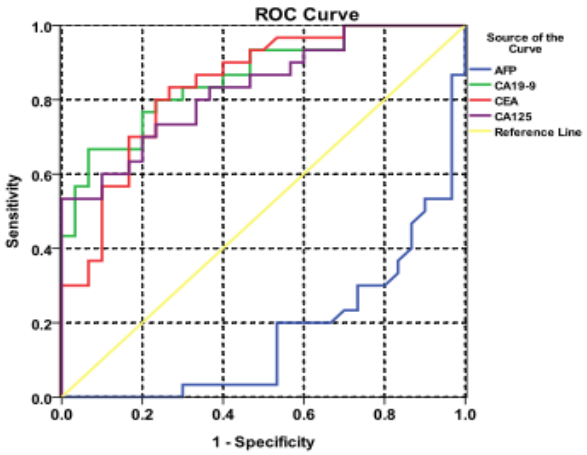

Figure 1. ROC Curve of the Single Detection of AFP, CA19-9, CA125 and CEA in the Diagnosis of CC

Table 3. Evaluation of Single Detection of AFP, CA19-9, CA125 and CEA in the Diagnosis of CC (%)

	Cut off value	Sensitivit	Specificity	Correct diagnosis (%)
AFP	15.40	100 (30/30)	0.00 (0/30)	50.00 (30/60)
CA19-9	125.07	76.67 (23/30)	80.00 (24/30)	78.33 (47/60)
CA125	95.65	66.67 (20/30)	83.33 (25/30)	75.00 (45/60)
CEA	12.05	53.33 (16/30)	86.67 (26/30)	70.00 (42/60)

The area under ROC curve of the single detection of serum CA19-9, CA125 and CEA is > 0.5, with sequence of CA19-9>CA125>CEA, but the area under ROC curve of the single detection of serum AFP was under diagonal reference line, <0.5, showing that the single detection of serum AFP was not of great significance to the differential diagnosis of CC (Table 2 and Figure 1).

According to ROC curve, the optimal cutoff values of serum AFP, CA19-9, CA125 and CEA were 15.40 ng/ml, 125.07 U/ml, 95.65 U/ml and 25.90 ng/ml, and of 4 indexes, the diagnostic effect of serum CA19-9 was the highest one for differential diagnosis of CC. With reference to CA19-9>125.07 U/ml, the sensitivity and specificity of the single detection were 76.67% and 80.00% and the rate of correct diagnosis was 78.33%. The diagnostic effect of CA125 is secondary to it and with reference to CA125>95.65 U/ml, the sensitivity and specificity of the single detection were 66.67% and 83.33% and the rate of correct diagnosis was 75.00%. With reference to CEA>12.05 ng/ml, the sensitivity and specificity of the single detection were 53.33% and 86.67%, with the rate of correct diagnosis of 70.00%. The diagnostic effect of AFP is worst and with reference to AFP>15.40 ng/ml, the diagnostic effect of CC and HCC patients was similar, with the sensitivity and specificity being 100.00% and 0.00%. By the above results, the sensitivity of serum AFP

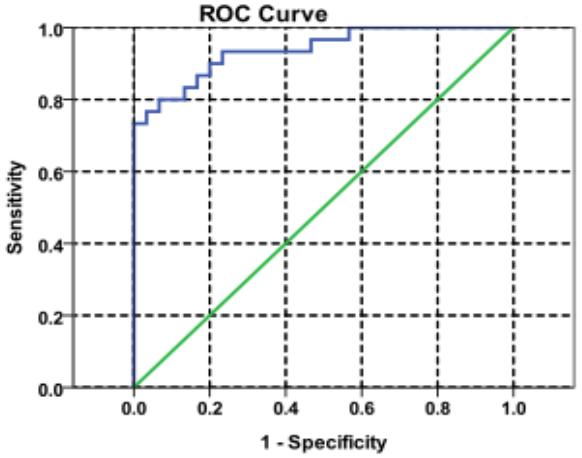

Figure 2. ROC Curve of Joint Detection of AFP, CA19-9, CA125 and CEA in the Diagnosis of CC

was highest and the specificity of CEA was highest, and the percentage correct of both CA125 and CA19-9 was 76.70% (Table 3).

Evaluation of diagnostic effect of joint detection of serum AFP, CA19-9, CA125 and CEA

The sensitivity, specificity and diagnostic accuracy of joint detection of AFP, CA19-9, CA125 and CEA was highest, the area under ROC curve was 0.94 (95%CI 0.88~0.99). It followed that joint detection of 4 indexes could greatly improve the diagnosis effect for CC and HCC (Table 4 and Figure 2).

Discussion

HCC and CC are generally similar in clinical manifestations, such as abdominal pain, abdominal distension and round or round-like masses in the liver. It is hard to diagnose by clinical manifestations. Although medical imaging examination is valuable for the diagnosis of a variety of diseases in clinic, medical imaging findings of CC is quite similar to HCC, which brings a big difficulty for clinical diagnosis (Asayama et al., 2015). Therefore, the golden standard of clinical diagnosis for CC is still postoperative pathological biopsy. Generally, distant metastasis of cancer cells is identified when they are diagnosed as CC. In recent years, with the development of medical laboratory science and the increasing cognition of medical specialists to tumors, the detection of the levels of serum tumor markers have become the new testing indexes for identification of early CC. Therefore, the detection of tumor markers has an important value on the clinical differential diagnosis, evaluation of efficacy and prognosis and followup of patients with tumors.

Tumor markers are a kind of substances which are produced by cancer cells or by the response of the body to cancer cells during the process of cancer cell production and proliferation, and accurately reflex the existence and growth of cancer cells (Liu et al., 2015). Tumor markers mainly include 6 kinks, namely, embryo-fetal antigens, carbohydrate antigens, hormones, enzyme and isoenzymes, proteins and cancer gene products. The proposal of tumor markers provides the possible approaches to the early detection of malignant tumors

Table 4. Evaluation of Diagnostic Effect of Joint Detection of AFP, CA19-9, CA125 and CEA in the Diagnosis of CC (%)

Marker	Sensitivity	Specificity	Percentage Correct
AFP+CA19-9	86.67 (26/30)	83.33 (25/30)	85.00 (51/60)
AFP+CA125	80.00 (24/30)	76.67 (23/30)	78.33 (47/60)
AFP+CEA	83.33 (25/30)	86.67 (26/30)	85.00 (51/60)
CA19-9 +CA125	76.67 (23/30)	90.00 (27/30)	83.33 (50/60)
CA19-9+CEA	76.67 (23/30)	83.33 (25/30)	80.00 (48/60)
CA125+CEA	80.00 (24/30)	76.67 (23/30)	78.33 (47/60)
AFP+CA19 -9+CEA	90.00 (27/30)	86.67 (26/30)	88.33 (53/60)
AFP+CA125 +CEA	83.33 (25/30)	86.67 (26/30)	85.00 (51/60)
CA19-9 +CA125+CEAe	80.00 (24/30)	90.00 (27/30)	85.00 (51/60)
AFP+CEA +CA19-9+CA125	90.00 (27/30)	90.00 (27/30)	90.00 (54/60)

(Shao et al., 2013).

AFP is a present best diagnostic index of specific to the diagnosis of early HCC which reflexes the changes of diseases and therapeutic effect of the patients and its specificity is secondary to pathological examinations. Taketa et al studied hepatopath (including HCC, liver cirrhosis and chronic hepatitis) in a hospital in Thailand and held that the best critical value of serum AFP was 200 ng/mL, with the sensitivity and specificity being 70% and 100%, respectively (Taketa et al., 2002). In this study, the results of serum AFP showed high specificity in the diagnosis of HCC and low in the diagnosis of CC.

CA19-9 is macromolecule glycoprotein whose level increases with the deterioration of diseases and decreases with the improvement of diseases. Based on this, the level of serum CA19-9 is usually considered as the detecting index of tumor-associated antigen (TAA), capable of accurately reflecting the confirmed state of gastrointestinal tumors, gastrointestinal tumors and CC, dynamic changes, prognosis and recurrence of tumors during the treatment (Chaiteerakij et al., 2014). CA19-9 is differentially expressed in patients with different types of PLC, and obviously highly expressed in CC patients, but is almost undetectable in HCC, which helps identification of HCC and CC. Patel et al (Patel et al., 2000) analyzed the level of serum CA19-9 in patients with benign and malignant biliary diseases and found that when the level of serum CA19-9 is >100 U/mL, the sensitivity of CC, benign liver diseases and biliary stricture were 53%, 24% and 8%, respectively, and the concentration of CA19-9 in patients with tumor resection was obviously lower than that in patients without tumor resection. Hence, CA19-9 may be the effective tumor marker of diagnosis of CC and monitoring the efficacy. Research has shown that the sensitivity of CA19-9, which not affected by age, gender, blood types, tumor location, differentiated degree of tumor of the patients, is reliable tumor marker in the diagnosis of CC (Tang et al., 2014). It is worth noting that the level of CA19-9 varies in different types of liver cancer in patients with PLC, even serum CA19-9 is valuable for monitoring the postoperative reoccurrence of CC. The

study of Kim et al showed that the overall survival of CC patients with postoperative CA19-9>37 U/mL is short (Kim et al., 2015). In this study, we also found that the diagnostic effect of CA19-9 is the highest one of 4 tumor markers in the diagnosis of CC.

CA125 is a macromolecule glycoprotein of molecular weight of 200~1 000 KD, synthesized in small intestine goblet cells of fetus, biliopancreatic epithelial cells as well as tumors such as pancreatic cancer in adults, gastric cancer, colon cancer. CA125 is mainly applied in the early diagnosis of pancreatic cancer and has the differential diagnosis value for some benign and malignant digestive tumors (Zuckerman et al., 1999; Charatcharoenwitthaya et al., 2008).

CEA is a sort of glycoprotein of complex structure separated from colon cancer tissues, with solubility. Generally, CEA exists in gastrointestinal tract, liver and pancreas tissues in embryonic period, and the level of it decreases after the birth. CEA, a common broad-spectrum tumor marker, elevates in multiple tumors such as CC. Nanashima et al thought that the high level of serum CEA is the key factor of poor prognosis of the patients, so the detection of serum CEA is of great importance for the evaluation of the prognosis of patients with intrahepatic cholangiocarcinoma (ICC) (Nanashima et al., 2007). Nakeeb et al explored the expression of bile CEA in biliary tract diseases and found that the level of bile CEA in patients with malignant biliary stricture was obviously higher than patients with benign biliary stricture, and increased gradually in progressive stage in patients with CC with the worsening of disease, bile CEA after tumor resection dropped dramatically to the normal range (Nakeeb et al., 1996). A study suggested that CEA was highly sensitive to the reoccurrence and metastasis of tumors, and by combined other tumor markers, applicable for judgment of benign and malignant diseases of liver and biliary system, and evaluation of severity degree and postoperative recovery of tumors (Sheen-Chen et al., 2007). However, the diagnostic sensitivity of CEA is susceptible to multiple factors (Tang et al., 2014). Therefore, CEA as a broad-spectrum tumor marker, cannot be regarded as the diagnostic index of tumor localization, but often as the index of observation of clinical efficacy and postoperative follow-up, and of improving diagnostic sensitivity and specificity by combined with other tumors (Tang et al., 2014).

In recent years, scholars advocates application of joint dynamic detection which makes up the shortness of the single detection and improves the detection rate, diagnostic sensitivity and specificity of liver and biliary system (Lumachi et al., 2014). In this study, the joint detection of AFP, CA19-9, CA125 and CEA meets the requirements of the elevating sensitivity, not obviously decreased specificity and the high accuracy of diagnosis; conform to the economical requirements, thus avoiding the unnecessary economic burden of the patients.

In conclusion, the detection of AFP, CA19-9, CA125 and CEA is of significance for the identification and diagnosis of CC and HCC. The diagnostic effect of CA19-9 for CC is superior to the other 3 tumor markers and the joint detection of 4 tumor markers can improve the diagnostic effect of CC and HCC.

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