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Serum CEA, CA125, CA19-9, and CA724 levels for the diagnosis and staging of cholangiocarcinoma.

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Abstract

Background: Aims serum markers provide a non-invasive, cheap and effective diagnostic and prognostic tool for cholangiocarcinoma. In this study, we aimed to evaluate serum levels of CEA, CA125, CA19-9 and CA724 for the diagnosis and staging of cholangiocarcinoma.
Materials and methods: Serum levels of CA125, CA19-9, CA724 and CEA were measured preoperatively, postoperatively and during follow-up in 153 cases of cholangiocarcinoma and 65 cases of benign biliary disease.
Results: Serum levels of these tumor markers elevated in cholangiocarcinoma, and CA19-9 was better than CEA, CA125, CA724 or the combination to diagnose cholangiocarcinoma with the cut off value of 73.25 U/ml (sensitivity 69.30%; specificity 87.7%). Serum CA125 and CA19-9 levels were helpful to assess advanced TNM stage.
Conclusion: Serum CA19-9 level is superior to CEA, CA125 or CA724 levels or the combination for the diagnosis of cholangiocarcinoma. Preoperative serum levels of CA19-9 could be used to predict advanced TNM stage and evaluate the resectability of cholangiocarcinoma.

Keywords: Cholangiocarcinoma, Tumor marker, Carcinoembryonic antigen (CEA), CA125, CA19-9, CA724.

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Introduction

Cholangiocarcinoma (CCA) is a rare malignant tumor originating from biliary tract epithelium [1]. CCA can be classified into Intrahepatic Cholangiocarcinoma (ICC) and Extrahepatic Cholangiocarcinoma (ECC), and ECC could be subdivided into Perihilar Cholangiocarcinoma (PCC) and Distal Cholangiocarcinoma (DCC) [2]. Painless obstruction accompanied with pale stools, dark urine and jaundice are common symptoms of CCA [3].

Carcinoembryonic Antigen (CEA) and Carbohydrate Antigen 19-9 (CA19-9) are two markers for the progression and recurrence of CCA with a wide variation in sensitivity and specificity [4,5]. Elevated CA19-9 level could predict malignant gastrointestinal cancers, and increased CA19-9 level is related to the prognosis of CCA [6-8]. CEA is a glycoprotein tumor marker relevant to colon and pancreas cancers and CCA [9,10]. CA125 presents an independent prognostic factor of intrahepatic CCA [11]. CA724 showed satisfactory clinical usefulness in patients with gastric cancer, but its value for CCA diagnosis has been rarely reported [12]. Up to now, no study has comprehensively evaluated the value of CEA, CA125, CA19-9 and CA724 for the diagnosis, staging and prognosis of CCA. This study aimed to explore the values of these four markers for the diagnosis and staging of all subtypes of CCA.

Patients and Methods

Subjects

153 CCA patients were enrolled who had undergone surgery from January 2007 to October 2015. Sixty-five patients with benign biliary disease diagnosed by clinical, radiological, and laboratory criteria were enrolled as controls. The diagnoses were confirmed by histological or biopsy examination. R0 resection was defined as removing the tumours completely

while any residual tumor was considered non-R0 resection [5]. Exclusion criteria were: primary tumor originated from other non-biliary organs or emerged with other tumours; preoperative chemotherapy or radiation therapy; preoperative and postoperative CA19-9 values were <2 U/ml, indicating the patients could not synthesize CA19-9 [13,14]; death during hospitalization. All subjects provided signed consent, and the study was approved by Institute Ethics Committee.

Surgical procedures and pathological evaluation

Radical resection or palliative surgery was performed on all patients using laparoscopy or laparotomy. The staging of tumours was categorized based on American Joint Committee Cancer (AJCC) TNM staging [2]. Histological grade was judged as High grade (H), Middle grade (M), or Low grade (L) [13].

Biochemical analysis

Peripheral blood was collected and subjected to laboratory tests, including liver biochemistry and serum biomarker tests, preoperatively, postoperatively between 1 and 6 weeks, once every month over 6 months and every 3 months after surgery. Biliary drainage was applied before chemotherapy or during the measurement of tumor markers when the patients presented with obstructive jaundice. Patients with preoperative and postoperative CA19-9 values <2 U/ml were excluded as mentioned above. Serum levels of CA125, CA19-9, CA724 and CEA were detected by immunoassay analyser (Roche Diagnostics, IN, USA). Biochemical parameters including Glutamic-Pyruvic Transaminase (ALT), Total Bilirubin (TBIL), Direct Bilirubin (DBIL), γ-Glutamyltranspeptidase (γ-GGT) and Alkaline Phosphatase (ALP) were measured by HITACHI 7170 analyser (Hitachi, Tokyo, Japan). The levels were reported as ng/mL for CEA and U/ml for CA125, CA19-9 and CA724. Recommended cut-off values of serum levels of CEA, CA125, CA19-9, CA724 were 5 ng/ml, 35 U/ml, 34 U/ml and 7 U/ml, respectively.

Statistical analysis

Comparison of tumor markers according to CCA subtype, pathological staging, histological grading, and classification of PCC were analysed by Kruskal-Wallis test. Optimal cut off values were determined based on Receiver Operating Characteristic (ROC) curves, and the Areas Under the Curve (AUCs) with 95% Confidence Intervals (CIs) and Standard Errors (SEs) were calculated. All statistical analyses were conducted by using SPSS 17.0 software (SPSS, Chicago, IL, USA), and $P<0.05$ was considered as significant difference.

Results

Patient demographics

One hundred fifty three consecutive patients with CCA were eligible for this study: 13 (7.8%) patients were excluded from a total of 166 subjects due to 8 cases of non-secretion of CA19-9 (pre- and postoperative values, <2 U/ml), 2 cases of CCA plus other tumours and 3 deaths during postoperative hospitalization. Patients clinicopathological information is shown in Table 1. The main subtypes of CAA such as ICC, PCC, and DCC were found in 62 (40.5%), 73 (47.7%), and 18 (11.8%) patients, respectively, with the TNM stages of I (5.8%), II (17.6%), III (22.9%), and IV (53.6%) and three histological grades of Highly Differentiated (HD 29.4%), Moderately Differentiated (MD 40.5%), Poorly Differentiated (PD 30.1%). Another four subtypes that provided important clues on surgical procedures according to the Bismuth classification of TNM staging were subdivided from 73 PCC patients as follows: I (2.7%), II (11.0%), III (31.5%), and IV (54.8%).

Clinical data

Preoperative levels of serum markers and biochemical indexes were described in the median and interquartile range shown in Table 2. Serum CA19-9 levels were the highest in malignancies (158.8 U/ml) followed by CA125 levels (19.30 U/ml), CEA levels (3.20 U/ml) and CA724 levels (2.60 U/ml) ($P<0.001$). The biochemical indexes of ALT, total bilirubin, direct bilirubin, GGT and ALP of the malignancies showed significant differences compared to benign patients ($P<0.005$).

Table 1. *Demographics and characteristics of all subjects.*

CCA (n=153)	
Median age, years (range)	59 (26-88)
Gender (man/woman)	86:67
CCA subtypes (n)	
ICC	62
PCC	73
DCC	18
TNM stage (n)[a]	
I	9
II	27
III	35
IV	82
Classification of CCA (n)[a]	
I	2
II	8
III	23
IV	40
Grade (n)	
H	45
M	62
L	46

Benign biliary diseases (n=65)	
Median age, years (range)	58 (18-88)
Gender (man/woman)	28:37
Diseases subtypes (n)	
Cholelithiasis (Hepatolithiasis, cholecystolithiasis, choledocholithiasis)	42
cholangitis	10
Reflux cholangitis	4
primary sclerosing cholangitis	3
autoimmune cholangitis	2
Biliary stricture	2
Bravery manager foreign body	1
Cholangiectasis	1

[a]According to the American Joint Committee Cancer (AJCC). TNM staging (7th edition, 2010). CCA: Cholangiocarcinoma; ICC: Intrahepatic Cholangiocarcinoma; PCC: Perihilar Cholangiocarcinoma; DCC: Distal Cholangiocarcinoma. H: High; M: Middle; L: Low.

Table 2. *Serum CEA, CA125, CA199 and CA724 levels and biochemical indexes.*

	Benign median (Quartile)	**CCA median (Quartile)**	**P value**
CEA	1.60 (0.95, 2.30)	3.20 (1.90, 6.30)	<0.001
CA125	10.30 (6.10, 21.75)	19.30 (10.80, 43.50)	<0.001
CA199	17.60 (9.70, 35.95)	158.80 (37.85, 658.30)	<0.001
CA724	1.20 (0.90, 2.00)	2.60 (1.30, 5.05)	<0.001
ALT	29.00 (16.50, 84.35)	60.00 (23.50, 162.00)	0.005
TBIL	16.40 (11.50, 31.55)	52.20 (13.15, 195.75)	<0.001
DBIL	5.05 (2.95, 12.00)	42.00 (5.35, 159.10)	<0.001
GGT	98.00 (38.50, 265.50)	217.00 (76.50, 586.00)	0.001
ALP	126.00 (80.00, 202.50)	240.00 (109.50, 420.00)	<0.001

CCA: Cholangiocarcinoma; ALT: Glutamic-Pyruvic Transaminase; TBIL: Total Bilirubin; DBIL: Direct Bilirubin; γ-GGT: γ-Glutamyltranspeptidase; ALP: Alkaline Phosphatase.

Characteristics of tumor location, TNM stage, histological grade and resectability of cholangiocarcinoma

The tumor stage was significantly associated with serum levels of CA19-9 and CA125 (P<0.001), but not of CEA or CA724 (P<0.43) shown in Table 3. The medians (interquartile concentrations) of serum CA125 levels were 11.30 (10.05, 17.70), 12.50 (10.30, 20.50), 18.90 (9.40, 32.50), and 27.15 (13.93, 67.05) U/mL for stage I, II, III and IV, respectively (P<0.001); Serum CA19-9 levels were 29.30 (9.60, 97.45), 125.60 (31.90, 311.50), 196.00 (34.60, 636.60), and 208.90 (65.13, 1788.00) U/mL, respectively, for stage I, II, III and IV (p<0.004).

In this study, 135 patients underwent R0 resection, and other 18 patients received non-R0 resection due to aggressive tumor behaviour. Patients who received non-R0 resection had elevated preoperative CA19-9 levels compared to patients who received R0 resection (P<0.05), whereas the increase in preoperative CEA, CA125 and CA724 levels in these two groups was not significant (P>0.05). Furthermore, we found no significant differences in serum levels among CCA subtypes (ICC, PCC and DCC) (P>0.930), histological grades (highly differentiated, moderately differentiated and poorly differentiated) (P>0.15) and PCC classification (Bismuth: I, II, III, IV) (P>0.25), indicating that serum levels of these markers are not affected by tumor location, histological grade or PCC classification (P>0.05) shown in Table 3.

Table 3. *Tumor location, TNM stage, histological grade and resectability of CCA.*

	No	**CEA**		**CA125**		**CA199**		**CA724**	
		Median (Quartile)	**P value**	**Median (Quartile)**	**P value**	**Median (Quartile)**	**P value**	**Median (Quartile)**	**P value**
CCA Subtypes									
ICC	62	3.35 (1.88, 7.36)	0.737[a]	21.95 (13.93, 53.85)	0.082[a]	102.75 (10.08, 695.38)	0.148[a]	2.35 (1.20, 6.18)	0.930[a]
PCC	73	3.10 (1.80, 5.90)		19.30 (10.05, 43.75)		198.90 (79.50, 1087.50)		3.00 (1.48, 4.75)	
DCC	18	3.80 (2.28, 6.10)		12.65 (10.53, 26.58)		130.30 (67.78, 288.25)		3.05 (1.25, 5.30)	
TNM stage									
I	9	2.30 (1.15, 4.90)	0.430[a]	11.30 (10.05, 17.70)	<0.001[a]	29.30 (9.60, 97.45)	0.004[a]	1.80 (0.85, 3.80)	0.082[a]
II	27	3.00 (1.90, 4.40)		12.50 (10.30, 20.50)		125.60 (31.90, 311.50)		3.30 (1.30, 5.80)	
III	35	3.60 (2.10, 6.30)		18.90 (9.40, 32.50)		196.00 (34.60, 636.60)		1.70 (1.05, 3.60)	

IV	82	3.50 (1.88, 7.55)		27.15 (13.93, 67.05)		208.90 (65.13, 1788.00)		3.00 (1.50, 6.05)	
Classification of PCC									
I	2	2.45 (1.80, 3.10)	0.676[a]	20.40 (10.80, 30.00)	0.702[a]	49.45 (8.10, 90.80)	0.358[a]	1.05 (0.50, 1.60)	0.083[a]
II	8	2.75 (1.50, 5.95)		12.80 (7.15, 23.82)		360.30 (74.25, 1139.78)		2.35 (1.33, 3.58)	
III	23	2.60 (1.30, 6.30)		14.40 (7.70, 52.80)		189.40 (29.30, 896.80)		2.00 (1.30, 4.10)	
IV	40	3.40 (2.25, 4.71)		21.70 (10.68, 44.88)		201.10 (100.95, 1699.75)		3.30 (1.85, 5.94)	
Grade									
L	46	3.55 (2.20, 5.85)	0.695[a]	20.80 (12.10, 75.125)	0.213[a]	162.90 (34.38, 690.85)	0.872[a]	2.50 (1.26, 3.70)	0.467[a]
M	62	3.10 (1.70, 6.55)		19.85 (11.73, 45.10)		173.10 (30.30, 1839.75)		2.95 (1.38, 5.58)	
H	45	3.50 (1.65, 6.35)		16.40 (10.00, 31.25)		147.70 (43.10, 602.85)		2.40 (1.25, 9.70)	
Resection									
Non-R0	18	3.10 (2.58, 4.88)	0.931[b]	21.80 (13.03, 74.65)	0.138[b]	698.20 (82.48, 1602.20)	0.047[b]	2.50 (1.30, 6.07)	0.615[b]
R0	135	3.25 (1.90, 6.38)		18.95 (10.65, 39.30)		142.80 (31.75, 562.50)		2.85 (1.30, 5.08)	

[a]Kruskal-Wallos test; [b]Mann-Whitney U test. CCA: Cholangiocarcinoma; ICC: Intrahepatic Cholangiocarcinoma; PCC: Perihilar Cholangiocarcinoma; DCC: Distal Cholangiocarcinoma; R0: No tumor cells residual; Non-R0: Tumor residual; H: High; M: Middle; L: Low.

Diagnostic value of serum CEA, CA125, CA19-9, CA724 levels in CCA

ROC curves for CCA were conducted to determine the diagnosis efficiency of these four tumor markers. The cut-off values of the four tumor markers in CCA were as follows: 2.45 ng/ml for CEA (sensitivity, 66.0%; specificity, 81.5%), 11.75 U/ml for CA125 (sensitivity, 72.5%; specificity, 60%), 73.25 U/ml for CA19-9 (sensitivity, 69.30%; specificity, 87.7%) and 2.25 for CA724 (sensitivity, 54.9%; specificity, 83.1%) shown in Table 4. The ROC curves for CA19-9 according to TNM stage showed that the cut-off values of CA19-9 for stage II, III and IV were 66.7 U/ml, 71.4 U/ml, and 73.2 U/ml, respectively shown in Table 5 (P<0.001), but there was no significant difference between benign disease and stage I of CCA (P=0.427).

The Areas Under the Curve (AUCs) were 0.764 (95% CI: 0.699-2.45), 0.682 (95% CI: 0.64-10.60), 0.796 (95% CI: 0.732-73.25), and 0.725 (95% CI: 0.656-2.30) for CEA, CA125, CA199, and CA724, respectively. According to the AUC, CA19-9 was the best single candidate for the diagnosis of CCA with the highest specificity but lower sensitivity than CA125 (P<0.001). CEA+CA199+CA125+CA724 showed the highest sensitivity (94.1%) but the lowest specificity. For CEA +CA19-9 the AUC was 0.792 (95% CI: 0.722-0.862) and showed a relatively higher sensitivity, but the specificity was lower than CA19-9 alone shown in Table 4 (P<0.001).

Table 4. *The diagnostic value of serum CEA, CA125, CA19-9 and CA724 for CCA.*

Markers	AUC	SD	P	95% CI		Cut-off	Sensitivity (%)	Specificity (%)
				Lower bound	Upper bound			
CEA	0.764	0.033	<0.001	0.699	2.45	2.45	66.0	81.5
CA125	0.682	0.041	<0.001	0.601	10.60	11.75	72.5	60.0
CA199	0.796	0.033	<0.001	0.732	73.25	73.25	69.3	87.7
CA724	0.725	0.035	<0.001	0.656	2.30	2.25	54.9	83.1
CEA+CA125	0.674	0.042	<0.001	0.591	0.757	-	85.6	49.2
CEA+CA199	0.792	0.036	<0.001	0.722	0.862	-	83.0	75.4
CEA+CA724	0.762	0.038	<0.001	0.689	0.836	-	81.7	70.8
CA125+CA199	0.707	0.042	<0.001	0.626	0.789	-	87.6	53.8
CA125+CA724	0.686	0.042	<0.001	0.604	0.769	-	85.0	52.3
CA199+CA724	0.774	0.037	<0.001	0.703	0.846	-	81.0	73.8

CEA+CA125+CA199	0.684	0.043	<0.001	0.600	0.768	-	92.2	44.6
CEA+CA125+CA724	0.688	0.043	<0.001	0.605	0.772	-	91.5	46.2
CA125+CA199+CA724	0.689	0.043	<0.001	0.606	0.773	-	90.2	47.7
CEA+ CA199+CA724	0.768	0.039	<0.001	0.692	0.843	-	88.9	64.6
ALL	0.678	0.043	<0.001	0.593	0.763	-	94.1	41.5

AUC: Area Under the Curve; SD: Standard Error; 95% CI: 95% Confidence Interval.

Table 5. *The diagnosis value of CA19-9 in TNM stage.*

Stage	AUC	SE	P	95%CI		Cut-off	Sensitivity (%)	Specificity (%)
				Lower Bound	Upper Bound			
	0.582	0.114	0.427	0.359	0.806	29	55.6	73.8
II	0.758	0.062	<0.001	0.636	0.880	73.25	66.7	87.7
III	0.788	0.053	<0.001	0.683	0.893	80.45	71.4	87.7
IV	0.835	0.035	<0.001	0.767	0.903	75.90	73.2	87.7

AUC: Area Under the Curve; SD: Standard Error; 95% CI: 95% Confidence Interval.

Discussion

Early diagnosis is very important to prolong the survival of CCA patients. Radiographic examinations are essential for the diagnosis of CCA but they are invasive and expensive. Laboratory tests based on tumor markers are very helpful for the diagnosis of CCA. The most widely studied tumor markers are CEA and CA19-9, which have increased levels in CCA patients. However, CEA or CA19-9 alone is neither sensitive nor specific for CCA. Here we investigated the diagnostic value of serum levels of CEA, CA125, CA19-9, CA724 in 153 patients with different CCA subtypes and reported the first study to explore these four serum tumor markers comprehensively in CCA.

We found that serum levels of these four tumor markers and several biochemical indexes significantly elevated in cholangiocarcinoma compared to benign biliary tract diseases. Some studies reported that the elevation of tumor markers was positively correlated with increased ALT, bilirubin, GGT and ALP levels, which are generally induced by biliary obstruction [5,6]. In the current study, our data showed that serum levels of CEA, CA125, CA19-9 and CA724 showed no correlation with tumor location (CCA subtypes), Bismuth classification of PCC and pathology grade, whereas serum levels of CA125 and CA19-9 elevated in patients with advanced CCA (stage III and IV) and serum level of CA19-9 was higher in non-R0 resection patients, indicating that preoperative serum levels of CA125 and CA19-9 are related to lymph node and distant metastasis and the chance of resectability.

Furthermore, we examined the diagnosis effects of these four tumor markers as single agent or in combination by ROC curve analysis. The results showed that CA19-9 level had the largest AUC (0.769), the highest specificity and relative sufficient sensitivity that could be identified as an independent predictive factor for CCA. CA125 level was excellent to indicate malignant biliary disease but could not discriminate CCA from benign biliary diseases. CA724 level showed insufficient sensitivity to the diagnosis of CCA [15,16], and a similar poor diagnosis of CEA was recommended [17]. Although combining four markers could increase the sensitivity for the diagnosis (81.0-94.1%), the specificity was reduced remarkably (41.5-75.4%), suggesting that the diagnostic accuracy of the combination was not better than CA19-9 alone. The preoperative cut-off values of CA19-9 for CCA TNM stage were established in this study and the cut-off values of stage I did not surpass those of benign control. However, the number of patients diagnosed as stage I was rare (8 cases in this study), and larger samples are necessary to confirm diagnostic value of CA19-9 for CCA.

Conclusion

In conclusion, serum CA19-9 level is superior to CEA, CA125 and CA724 levels or the combination for the diagnosis of CCA. The preoperative levels of CA19-9 can be used to predict advanced TNM stage and evaluate the resectability of CCA.

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