

## Original Article

# Prognostic value of serum CA125 levels in cholangiocarcinoma

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**Abstract:** Cholangiocarcinoma (CCA) is an aggressive tumor characterized by distant metastasis and poor prognosis. Therefore, it is important to develop non-invasive and effective prognostic markers for CCA. This study aimed to evaluate prognostic value of serum CEA, CA125, CA19-9 and CA724 levels in CCA. Total 150 patients with CCA who underwent surgery at Wuhan General Hospital from January 2012 to December 2015 were enrolled as the study subjects. Serum levels of CEA, CA125, CA19-9 and CA724 were measured before surgery, after surgery and at follow-up. The factors predicting CCA recurrence were analyzed using multivariable regression analysis. The results showed that serum CA125 and CA19-9 levels could predict the recurrence of CCA with the cut-off values of 16.3 U/ml for CA125 and 34.6 U/ml for CA19-9 ( $P < 0.01$ ). In contrast, serum levels of CEA and CA724 were not correlated with CCA recurrence ( $P = 0.12$  and  $0.14$ ). The recurrence ratio showed no significant difference between patients with R0 resection who received postoperative adjuvant chemotherapy and those who did not receive chemotherapy ( $P > 0.05$ ). However, the patients who received adjuvant chemotherapy had significantly longer median recurrence-free time (10.2 months) than those who did not receive adjuvant chemotherapy (5.3 months). Multivariate regression analysis identified three factors significantly associated with the recurrence in CCA patients with R0 resection: adjuvant chemotherapy, TNM stage II, III and IV, and preoperative serum level of CA125. In conclusion, preoperative serum CA125 level can be used to predict CCA recurrence. Postoperative adjuvant chemotherapy can improve the recurrence-free survival of CCA patients. Further large-scale studies are needed to confirm prognostic value of serum CA125 level in CCA.

**Keywords:** Cholangiocarcinoma, biomarkers, prognosis, CEA, CA125, CA19-9, CA724

### Introduction

Cholangiocarcinoma (CCA) is a malignant tumor originating from the biliary tract epithelium with increasing incidence worldwide [1]. According to the 7<sup>th</sup> edition of American Joint Committee on Cancer (AJCC), CCA can be classified into intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC), and ECC is further divided into perihilar cholangiocarcinoma (PCC) and distal cholangiocarcinoma (DCC) [2]. CCA is an aggressive malignancy characterized by early lymph node involvement and distant metastasis, with 5-year survival rates of 5%-10% [3, 4]. Therefore, it is important to develop non-invasive and effective prognostic markers for CCA.

The identification of serum markers has served as non-invasive, cheap and effective prognos-

tic tool to help develop therapy strategies for CCA. Carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) are two important markers used to monitor tumor progression and recurrence in CCA [5, 6]. Increased serum CA19-9 level is related to the prognosis in patients with CCA [7]. CEA is a glycoprotein tumor marker recently implicated in the prognosis of CCA [8]. CA125 has an inadequate diagnostic specificity, but presents an independent prognostic factor of poor survival of intrahepatic CCA [9]. CA724 is another carbohydrate antigen proposed as a prognostic marker for gastric cancer [10], but the prognostic value of CA724 for CCA remains unclear.

In this study, we aimed to explore the prognostic value of serum markers CEA, CA125, CA19-9 and CA724 for CCA.

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**Table 1.** Clinicopathological characteristics of all subjects

CCA	N = 150
Median age (range), years	54.6 (30-84)
Gender (male/female)	79:71
CCA Subtype	
ICC	57
PCC	72
DCC	21
TNM stage	
I	7
II	25
III	37
IV	81
Grade	
High	39
Middle	59
Low	52

## Materials and methods

### Subjects

150 patients with CCA who underwent surgery at Wuhan General Hospital from January 2012 to December 2015 were enrolled as the study subjects. All data from the subjects were retrieved from hospital database and analyzed retrospectively. R0 resection indicated complete removing of the tumors and no tumor cells in the margins could be detected by microscopic examination; while non-R0 resection indicated residual tumor [6]. Subjects who met exclusion criteria were excluded: primary tumors originated from other non-biliary organs or emerged with other tumors; received chemotherapy or radiotherapy before surgery; preoperative serum CA19-9 level was less than 2 U/ml; death after operation. Written informed consent was obtained from all subjects, and the study was approved by Ethics Committee of Wuhan General Hospital.

### Surgical operation and pathological evaluation

Radical resection was performed using laparoscopy or laparotomy based on local tumor invasion, lymph node metastasis and pathological status. The stages of tumors were categorized according to American Joint Committee Cancer (AJCC) TNM staging (7<sup>th</sup> edition, 2010). The tumor histological grade was classified as

high, middle and low according to predominant pattern of histopathological differentiation [11].

### Measurement of serum CEA, CA125, CA19-9 and CA724 levels

Peripheral blood was collected from the patients preoperatively, postoperatively, once every month over 6 months and every 3 months thereafter after surgery. In control subjects, blood was collected when they were first admitted to the hospital. Serum levels of CEA, CA19-9 and CA724 were measured by electrochemiluminescence immunoassay using E170 MODULAR Immunoassay Analyzer (Roche Diagnostics, Rotkreuz, Switzerland), and serum CA125 level was measured by enzyme-linked immunosorbent assay (ELISA, CA125-ELISA-Kit, CanAg, Gothenburg, Sweden). Recommended cut-off levels were 5.0 ng/ml, 37.0 U/ml, 6.7 U/ml, and 20.0 U/ml for CEA, CA19-9, CA72-4, and CA125, respectively.

### Statistical analysis

Comparisons between groups were analyzed using the Mann-Whitney U test or Kruskal-Wallis test. The recurrence rate in R0 resection patients was calculated using Chi-squared test with the significance corrected by the Bonferroni method. The predictors for recurrence were analyzed using multivariable regression analysis, and hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated for each predictive factor. All statistical analyses were performed using SPSS 17.0 software (SPSS, Chicago, IL, USA).  $P < 0.05$  indicated significant significance.

## Results

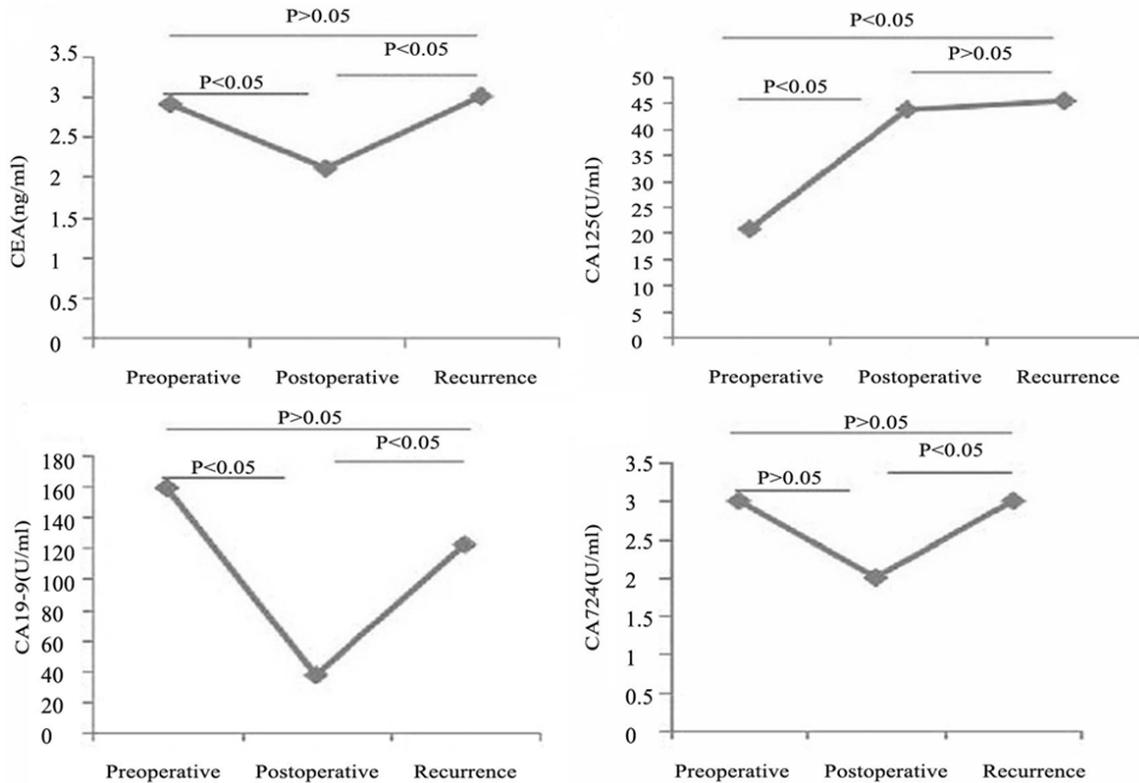
### Clinicopathological characteristics

Clinicopathological characteristics of all subjects were shown in **Table 1**.

### Prognostic value of serum CEA, CA125, CA19-9 and CA724 levels in CCA

Serum CEA, CA19-9 and CA724 levels were high before the operation but declined significantly after curative resection. However, Serum CEA, CA19-9 and CA724 levels recovered when the patients developed CCA recurrence ( $P <$

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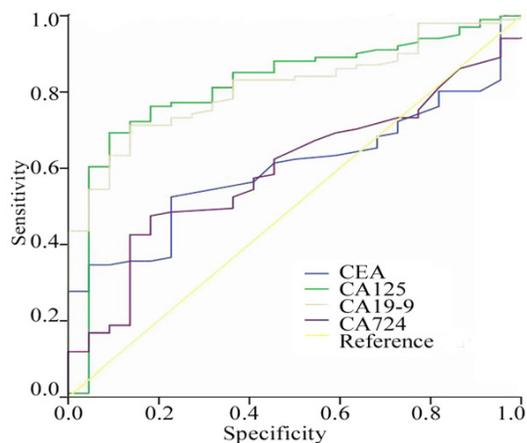


**Figure 1.** Serum levels of CEA, CA125, CA19-9, and CA724 before operation, after operation and during the recurrence of CCA.

**Table 2.** The value of CEA, CA125, CA19-9 and CA724 to predict CCA recurrence

Markers	AUC	SE	P value	95% Confidence Interval		Cut-off	Sensitivity (%)	Specificity (%)
				Lower Bound	Upper Bound			
CEA	0.601	0.054	0.12	0.496	0.706	4.98	34.7	95.9
CA125	0.818	0.050	< 0.01	0.720	0.915	16.5	69.3	90.9
CA19-9	0.813	0.041	< 0.01	0.733	0.894	34.75	71.3	86.4
CA724	0.597	0.059	0.14	0.481	0.712	3.05	47.5	81.8

AUC, area under the curve; SE, standard error; 95% CI, 95% confidence interval.



**Figure 2.** Serum levels of CEA, CA125, CA19-9, and CA724 for the prediction of CCA recurrence.

0.05) (**Figure 1**). In contrast, serum CA125 level increased significantly after operation compared to before operation, and showed no significant difference when the patients developed CCA recurrence ( $P > 0.05$ ) (**Figure 1**).

ROC curves were developed to determine the association of serum CEA, CA19-9 and CA724 levels and CCA recurrence. The results showed that only CA125 and CA19-9 could predict the recurrence with the cut-off values of 16.3 U/ml (sensitivity 69.3%; specificity 90.9%) for CA125 ( $P < 0.01$ ), and 34.6 U/ml (sensitivity 71.3%; specificity 86.4%) for CA19-9 ( $P < 0.01$ ). In contrast, serum levels of CEA and CA724 were not correlated with CCA recurrence ( $P = 0.12$  and  $0.14$ , **Table 2**; **Figure 2**).

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**Table 3.** Recurrence in CCA patients with R0 resection with or without post-operative chemotherapy

	Chemotherapy		Total
	No	Yes	
Non-recurrence	17	27	44
Recurrence	43	41	84
Total	60	68	128

**Table 4.** Multivariate analysis of factors affecting CCA recurrence

Factors	B	SE	Wald	df	P	Exp (B)	95.0% CI for Exp (B)	
							Lower	Upper
Stage <sup>a</sup>			8.823	3	0.032			
Stage (II)	0.007	0.575	0.000	1	0.990	1.007	0.326	3.106
Stage (III)	0.168	0.550	0.093	1	0.760	1.183	0.402	3.476
Stage (IV)	0.744	0.523	2.026	1	0.155	2.105	0.755	5.866
Chemotherapy	0.670	0.210	10.153	1	0.001	0.512	0.339	0.773
Preoperative CA125 level	0.002	0.001	3.455	1	0.063	1.002	1.000	1.005

<sup>a</sup>Stage II, III and IV compared to stage I; P, P value; Exp (B), hazard ratio; 95% CI, 95% confidence interval.

### Prognostic factors associated with CCA recurrence

We further analyzed the relationship between CCA recurrence and adjuvant chemotherapy in patients with R0 resection. Among 128 CCA patients who underwent R0 resection, recurrence was confirmed in 84 (65.6%) patients, and 41 patients with recurrence received post-operative adjuvant chemotherapy (Table 3). The recurrence ratio showed no significant difference between patients with R0 resection who received postoperative adjuvant chemotherapy and those who did not receive chemotherapy (P > 0.05). However, the median recurrence-free time was 10.2 months in the patients who received adjuvant chemotherapy but was 5.3 months in those who did not receive adjuvant chemotherapy. The recurrence-free time of patients who received adjuvant chemotherapy was significantly longer (log-rank test, P = 0.001).

Next, we employed a multivariate model to analyze prognostic factors associated with recurrence. According to the forward method of Cox regression, three factors were identified as affecting recurrence in CCA patients with R0 resection: adjuvant chemotherapy as a protective factor with an HR of 0.512 (95% CI: 0.339-

0.773; P < 0.001), TNM stage with HRs of 1.007, 1.183, and 2.105 for stages II, III and IV, respectively, and preoperative serum level of CA125 with an HR of 1.002 (Table 4).

### Discussion

CCA is a malignant tumor often diagnosed at advanced stage when it is characterized by local invasiveness and regional lymph node and distant metastasis. The unsatisfactory survival of CCA patients is a big challenge in the clinical.

Therefore, effective and non-invasive prognostic markers are urgently needed to develop therapeutic strategies to improve the survival of CCA patients. In this study, for the first time we investigated prognostic value of serum CA19-9, CEA, CA125 and CA724 levels in 150 patients with CCA.

Previous studies have reported several potential biomarkers for the prognosis of malignant biliary cancer [12-14]. However, prognostic markers of CCA have been rarely reported. In present study, we focused on the levels and cutoff values of tumor markers CA19-9, CEA, CA125 and CA724 in patients with CCA recurrence. We found that postoperative levels of CEA, CA19-9 and CA724 were significantly lower compared to preoperative levels, and then increased to high levels with the recurrence of CCA. However, serum CA125 level did not show significant difference before and after the recurrence. We performed ROC curve analysis and found that only serum CA125 and CA19-9 levels were useful to predict CCA recurrence, and the optimal cut-off values of serum CA125 and CA19-9 levels were 16.3 U/ml and 34.6 U/ml, respectively.

CA125 was reported as an independent prognostic predictor associated with poor survival

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in patients with ICC [9]. CA125 is secreted from the endometrium, fallopian tube, amniotic cells, lung and peritoneum, and CA125 level would increase under pathologic situations [15]. As CA125 is originated from the epithelium, surgery may be a promoting factor to stimulate the secretion of CA125 from the epithelium [16]. This may explain why serum CA125 level increased after surgery. Further studies involving large sample of CCA patients are needed to confirm the prognostic value of CA125 in CCA.

Adjuvant chemotherapy could improve the prognosis of patients with resectable CCA [16]. In this study, we compared the recurrence rate of CCA in patients with R0 resection who received adjunct chemotherapy and those who did not. A large part of the enrolled CCA patients underwent R0 resection (128/150, 85.3%). The recurrence rate was 71.7% in patients who did not receive chemotherapy and 60.3% in patients who received chemotherapy, and the difference in recurrent rate was not significant ( $P > 0.05$ ). However, recurrence-free time of patients who received adjuvant chemotherapy was significantly longer than that of patients without postoperative adjunct chemotherapy (10.2 months versus 5.3 months). These results suggest that adjuvant chemotherapy is an important factor to prolong the recurrence-free survival of CCA patients. Furthermore, multivariate analysis confirmed that adjuvant chemotherapy was a prognostic factor to prolong recurrence-free time in CCA patients with R0 section. In addition, high TNM stage and high preoperative serum CA125 level were correlated with increased risk of CCA recurrence.

In conclusion, preoperative serum CA125 level can be used to predict CCA recurrence, Postoperative adjuvant chemotherapy can improve the recurrence-free survival of CCA patients. Further large-scale studies are needed to confirm prognostic value of serum CA125 level in CCA.

### Disclosure of conflict of interest

None.

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